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Regional Distribution of Circulating Blood During Submersion Asphyxia in the Duck

By

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Abstract

Johansen, K. *Regional distribution of circulating blood during submersion asphyxia in the duck.* Acta physiol. scand. 1964. 62. 1—9. — Regional blood flow distribution in ducks was estimated from fractional distribution of Rb Cl. A comparison was made between normally breathing ducks and submerged ducks. The data document that conspicuous changes in regional blood flow take place upon submersion. The skin, skeletal muscle and organs of the gastrointestinal system showed a marked decrease in activity in the submerged condition down to an average of 3.9 for the gizzard, 29.4 for skin excised from the thorax and 11.0 for gastrocnemius muscle. Surprising exceptions to this were displayed by tissues in the cranial part of the animals. Thus both skin and muscle from the head region showed an increase in activity during submersion. The same was true for the excised eye. The esophagus similarly showed an increased activity in the submerged animals. The myocardium from both atria and ventricles showed a striking increase amounting to 4.1 times higher activity in the left ventricular myocardium in the submerged animals. The change in vasomotor constrictor tone taking place upon submersion is thus highly selective and possibly segmentally oriented giving an increase in peripheral resistance to most organs posterior to the heart while the most cranial tissues seem to be subjected to a general decrease in vasoconstrictor tone with an increased blood flow. Activity in the kidneys was markedly consistent and 9.1 of the value found in normally breathing animals. The adrenals showed an interesting increase in activity on the average as high as 4.9 times the value in normally breathing animals.

The exceptional endurance to asphyxia displayed by naturally diving animals has long been a focus of interest to physiologists. A marked cardiovascular response is without exception considered to be a key controlling factor in the endurance of the asphyctic condition in these animals. The line of reasoning suggests that oxygen is conserved for

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TABLE I demonstrates changes in organ activity comparing normally breathing (N) and sub-
the normally breathing subject is listed in a special column for each set of experi
are listed to the right in the table

Sample	I			II		
	N	S	%	N	S	
Skin						
Web	134	3 160	—	380	40	10 5
Thorax	3 641	1 883	51 1	3 971	401	10 1
Head	4 774	9 740	200	4 038	9 3?7	200
Muscle						
Gastrocnemius	4 830	608	12 5	5 6?4	50	1 0
Pectoralis	9 040	2 202	24 3	5,330	918	17 ?
Neck muscle	4 66?	1 650	35 3	3 000	560	18 6
Masseter	2 4??	3,230	130	2 505	7 300	290
Heart						
Left atrium	7 7?9	47 ?68	615	8 000	50 19?	627
Right ventricle	1? 69?	86 064	548	17 098	8?,200	492
Left ventricle	31 03?	140 037	450	27,810	105 648	380
Gastro-intest						
Esophagus	*2 320*	*13 473*	?90	*2 109*	*5,876*	*270*
Gizzard	? ?62	594	10 5	5 482	no act	–
Intestine	9 636	4 ?71	47 2	10 628	190	1 7
Glands						
Pancreas	7 600	1 175	15 4	8 8??	344	3 9
Liver	6 549	4,383	66 8	8 467	10 509	124
Adrenals	4 383	11,837	2?0	3 441	?0 000	581
Thyroid	3 90?	12 889	330	3 790	5 0?0	133
Kidney	91,220	14 1??	15 5	61 6?0	9 517	15 4
Spleen	4 9?8	10 844	?19	6 447	? 440	114
Eye	6 3??	20 340	310	4 ?05	14 7?2	3?0
Diencephalon	5?0	2,831	?14	?7	1,818	250
Heart rat (beats min)	1?0	1?	8.8	180	15	8 3

the most vital tissues by a redistribution of the circulating blood reducing flow through skin, muscle and splanchnic areas with a maintained or augmented flow to the central nervous system The only directly measured parameter of support to such a reasoning has been a profound bradycardia and a relatively unchanged systemic blood pressure (Irving 1939 Irving Scholander and Grinell 1941 Irving Scholander and Grinell 1942, Johansen and Krog 19?9 Feigl and Folkow 1963

A number of authors have reported indirect measures of a reduced blood flow through skin and skeletal muscle by local temperature recordings and accumulation

merged (S) animals expressed as counts per gram tissue per minute The per cent change from mental animals The heart rates are indicated at the bottom of each column. Average values

III			IV			Average		
N	S		N	S		N	S	
251	465	180	221	50	22 6	246	185	—
3 047	575	18 5	4 075	1 500	36 7	3 745	1 089	29 4
2 826	11 525	370	4 322	21 640	500	3 990	13 065	327
5 267	1 395	26 4	6 533	420	6 4	5 571	618	11 0
1 553	1 335	87 0	7 207	1 004	13 7	5 782	1 364	23 6
3 869	1 096	28 3	1 040	1 109	106	3 143	1 103	35 9
3 000	5 000	166	2 571	7 970	309	2 640	5 875	222
4 793	28 507	594	11 277	61 400	506	7 957	46 967	565
11 377	31 113	27 3	14 111	70 778	501	14 570	68 287	468
22 852	84,391	370	29 674	123 870	417	27 842	113 486	407
2 821	6 000	212	2 548	6 830	268	2 449	8 044	320
3 950	85	1 5	4 946	83	1 6	4 619	254	3 9
8 300	225	2 1	9 670	2 023	31 3	9 558	2 005	20 9
4 222	90	2 1	7 564	608	8 0	7 060	554	7 8
6 000	5 562	92 7	9 434	4 011	42 4	7 612	6 116	80 3
2 853	16 685	584	2 985	19 480	652	3 415	17 000	497
2 000	4 625	231	4 122	11 412	276	3 454	8 494	245
24 519	4 162	16 9	66 923	4 395	6 5	61 083	5 560	9 1
10 639	1 514	14 2	6 656	6 436	96 5	7 175	6 573	91 6
3 203	16 644	51 6	3 [illegible]03	15 000	405	4 374	16 676	381
380	1 184	31 1	485	1 158	238	535	1 747	326
200	15	7 5	210	15	7 1	190	15	7 9

of lactic acid in muscle (Scholander Irving and Grinell 1942a Scholander Irving and Grinell 1942b Andersen 1959) by thermostromuhr technique (Irving 1938b) or a modified plethysmographic venous occlusion method (Johansen and Krog 1959) More recently Hollenberg and Uvnas (1963) have provided more direct evidence of blood flow changes in skin muscle and splanchnic areas by using a drop flow meter technique The present study provides instantaneous values of regional blood flow distribution from a wide variety of tissues in unanesthetized ducks by using the technique described by Sapirstein (1958) based on fractional distribution of a radioactive indicator

Materials and methods

Domestic ducks were used as experimental animals The animals were immobilized in a canvas jacket The feet the neck and head and the right wing protruded out through the restraining jacket The right brachial vein was exposed and cannulated under local anesthesia The catheter (PE 50) was advanced to the proximity of the right atrium Electrocardiographic leads were mounted subcutaneously and extended to an Elema Schonander electrocardiograph Submersion was done by manually placing the animals head in a water container Care was exercised to avoid major postural changes of the animal during submersion The method used to measure regional blood flow distribution follows in all essentials the description by Sapirstein (1958) The method has earlier found widespread applications by a number of workers (Hershgold Steiner and Sapirstein 1959 Levy and Martins De Oliveira 1961 Steiner and Mueller 1961 Johansen 1961 Bullard and Funkhouser 1962) The basis of the method is that the uptake of an indicator by an organ is related to the organ blood flow the arterial concentration of the indicator and the organs extraction ratio for the indicator When Rb^{86} Cl is introduced into the blood stream it will readily distribute into the intracellular compartment and the concentration of Rb in a certain tissue will be proportional to the fraction of the cardiac output reaching that tissue According to Sapirstein (1958) the amount of indicator deposited will remain in the tissue for a certain time Sapirstein found a uniform extraction ratio for all organs except the brain for periods up to a minute

In the normally breathing ducks the heart rate values ranged from 170 to 210 beats/minute After injection of 10 μc Rb Cl in 2 ml saline into the brachial vein catheter the heart was stopped momentarily 50 sec later by injection of a saturated potassium chloride solution into the venous catheter Postmortem blood was withdrawn for determination of remaining activity Subsequently the animal was dissected and the various tissues excised and their activity counted A Tracer lab scintillation counter was used together with a Tracer lab scaler to record the activity Tissues were counted for 5 min at 1 900 v In the submerged ducks the indicator was injected when the heart rates had reached a steady level of approximately 15 beats/min The heart was stopped after approximately 2 min by injection of the saturated potassium chloride solution The time dependence of the indicator concentration in various tissues in the diving ducks was found to stay unchanged from about 40 sec to more than 2 min after injection of the indicator The rest of the procedure followed what is described for the normally breathing ducks In the present study only relative values of regional blood flow distribution in the two experimental situations were sought and no integrations done of the arterial concentration after injection

Results

Table I gives a composite picture of the results obtained in the present investigation expressed as counts/g tissue/min The per cent change in going from normal breathing to submersion is listed in a special column for each set of the experimental animals Average values are found to the far right of the table The heart rate during normal breathing and submersion is indicated at the bottom of each column Figure 1 shows the percentage of total injected activity deposited per gram tissue in a number of different tissues comparing the normally breathing animals (black bars in the diagram) *with the submerged animals*

Samples of skin were taken from the web of the foot from the thorax and the head region Activity in the web skin varied greatly seemingly unrelated to changes induced by the submersion It was readily seen that circulation through the web is dominantly governed by temperature regulative requirements If an animal became subjected to a positive heat load as was the case of the submerged animal I the heat dissipating requirements completely overruled any possible constrictive effects of submersion asphyxia Circulation through skin from the thorax region which is entirely covered by feathers and presumably plays no part in temperature regulation showed a uniform vasoconstrictor response to the submersion asphyxia There was a markedly reduced activity on the

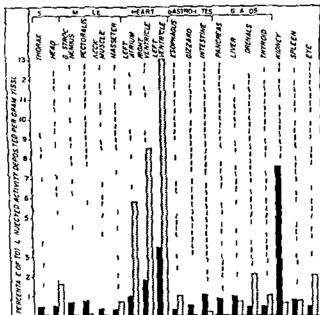


Fig 1 Percentage of total injected activity of Rb deposited per gram tissue in various organs of domestic ducks The black bars indicate values during normal breathing the striped bars values obtained during submersion The graph represents average values from eight ducks

average about 30% of the values for the normally breathing animals However skin excised from the head region showed a consistent increase in activity ranging from 2 to 5 times the value found in normally breathing animals with an average increase of 3 2 times (Fig 1)

Muscle samples were similarly selected along the long axis of the body in order to detect any possible flow changes related to an anterio-posterior localization of the tissue Samples of the gastrocnemius muscle showed a strikingly uniform reduction in activity In one submerged animal (no II) the activity was as low as to approach the background value The average value for activity during submersion was only 1/10th of the values from normally breathing animals Samples from the strongly developed pectoral muscle showed the same general reduction in activity however somewhat less prominent but still down to less than 1/4th of the values in normally breathing animals Skeletal muscle from the lower neck region showed changes parallel to those from the pectoral muscle In one animal (no IV) there was an insignificant change from the values in normally breathing animals Skin and muscles from the head region for example the masseter muscle displayed a completely different picture with an average increase of 2 2 times in activity during submersion (Fig 1) From the heart region samples were excised from the left atrium and from the right and left ventricle All these tissues showed a profound increase in activity during submersion reflecting marked changes in the underlying blood flow (Fig 1) Descending down the gastro-intestinal tract revealed results with the same general tendency as those obtained from skin and

skeletal muscle Thus did samples from the esophagus show a considerable increase in activity during submersion ranging from about 6 0 times in animal I to about 2 7 times in animal III The average increase was 3 2 times the value in the normally breathing animals Samples from the gizzard however taken a few centimeters more caudally showed a conspicuous reduction in activity In animal II the activity was practically similar to that of the background The average submersion value was as low as 3 9 % *of the activity in the normally breathing animals The samples taken from the small* intestine showed a varied but significant reduction during submersion with values ranging from 47 2 % to 1 7 % and with an average of 20 9 % of the values found in normally breathing animals Samples from a lower segment of the intestines (not shown in the table) revealed a completely parallel picture The pancreas samples taken from the submerged animals were uniformly low and on the average 7 8 % of the activity in normally breathing animals The liver on the other hand showed a more moderate reduction during submersion with average activity values of 80 3 % of the average value for normally breathing ducks

The adrenal gland showed an interesting response with a consistent increase in activity The increase was indeed considerable ranging from 2 7 to 6 5 times that of normally breathing ducks The average increase was 4 9 times and second only to the increase in left atrial activity (Fig 1) The thyroid showed a similar but less conspicuous increase of 2 4 times during submersion (Fig 1) The kidneys were suspected to demonstrate striking changes in the submerged animals and the activity in the kidney samples revealed a reduction in underlying blood flow during submersion down to an average of 9 1 % of the values in normally breathing animals The various samples showed a very uniform response (Table I) The spleen on the other hand showed the greatest constancy in activity of all organs tested comparing normally breathing and submerged animals with an average during submersion of 91 6 % of the activity in the normally breathing animals The conspicuous changes related to the anterio-posterior *orientation of the organs and tissues suggested that other more specialized organs were* tested The eyes were excised from all test animals and showed an increase in activity of 3 3 times during submersion As earlier emphasized by Sapirstein (1958) in developing and evaluating the method used presently the central nervous system does not render itself for analysis by the method In spite of the fact that the counts obtained from central nervous tissue are thus unreliable and meaningless as compared with other tissues from the same subject it was felt that a comparison between the two groups of subjects might reveal suggestive information about the relative blood flow changes in the normal breathing and submerged condition Such analysis revealed an increase in flow through the diencephalon 3 times the average value in the normally breathing animals

Discussion

It has for obvious reasons been difficult to obtain comprehensive data on the partition of the cardiac output in unanesthetized animals and in particular in animals that undergo a regime of experimental submersion The results here presented makes possible a more direct and detailed analysis of the conspicuous redistribution of blood flow which has been alleged to occur in many animals upon submersion It should be inferred however that th method used of fractional dispersion of an indicator has the disadvantage of measuring regional blood flow at only a given instant in a single animal

For this reason rigid precautions were taken to standardize the experimental circumstances and to use experimental animals as similar as they could possibly be selected. Irving (1939) was the first to propose the thesis that a dominant factor in the success of naturally diving animals in their endurance of prolonged asphyxia consists in a redistribution of blood flow and thereby of available oxygen to maintain the integrated activity of the central nervous system and allowing less sensitive tissues with a better capacity for anaerobic metabolism to do with less oxygen. Most workers in the field have provided support for this idea. Thus Irving himself (1938b) showed with thermostromuhr technique in a number of different species even including typical "non-divers" like cats and dogs that circulation decreased markedly through skeletal muscles but was maintained or increased in the brain during apnea. He also showed that the changes in muscle blood flow resulted from increased activity in sympathetic vasoconstrictor fibers. Scholander (1940) detected an accumulation of lactic acid in skeletal muscle in seals which was not released to the circulating blood until after the dive. He presents this as evidence for muscle ischemia during diving. Grinnell, Irving and Scholander (1942) deduced a muscular vasoconstriction in seals by heat conductivity methods. Andersen (1959) extended these studies to ducks and got similar results. Eliassen (1960) takes exception from all other workers and maintains that muscular blood flow does not change in response to submersion of avian divers. As pointed out by Scholander *et al.* (1962) the data of Eliassen (1960) are open to serious criticisms because of methodical deficiencies. More recently Hollenberg and Uvnäs (1963) have measured regional blood flow through skin, muscles and splanchnic areas by a drop flow meter technique. These authors argue that progressive asphyxia will stimulate chemo-receptors and produce an increase in peripheral resistance and an early and progressive fall in heart rate. They support the concept that blood flow redistribution is important in the toleration to prolonged asphyxia displayed by diving animals. They documented a marked fall in the splanchnic and skin blood flow during submersion but were unable to demonstrate any marked change in skeletal muscle blood flow. It can be argued that the method employed by these workers is rather crude in respect to retaining a normal condition of the animal. For example, the skin was excised from the leg of the duck in which muscular blood flow was measured.

Reports on regional blood flow changes during submersion to organs other than skin, muscles and the splanchnic area are not available in the literature apart from a report by Irving *et al.* (1942) about a marked decrease in mesenteric blood flow and observations by Murdaugh *et al.* (1961) showing that the renal blood flow practically stops during a dive in seals.

The data acquired presently bring indisputable support to the idea that skeletal muscular blood flow is radically reduced during submersion in ducks. It seems, however, highly significant that the muscles of the head show a deviating response and a consistent increase in perfusion during submersion. The fact that skin and tissues along the gastro-intestinal tract show a similar response related to an antero-posterior localization of the tissue justifies the assumption that the increase in sympathetic vasoconstrictor outflow during submersion is highly selective and perhaps segmentally oriented. The finding that the esophagus shows a markedly increased activity while the gizzard located a few cm more posteriorly is reduced to 3.9 ... in activity during submersion points to a striking differentiation in the sympathetic outflow from the spinal nerves. The author contends that while the sympathetic vasoconstrictor tonus increases to the main bulk of skin and skeletal muscle and most visceral organs during submersion

it actually decreases to the heart and to several tissues and organs cranial to the level of the heart This finding seems at first hand to be exceptional as the general concepts about an increased sympathetic discharge in the vasomotor nerves involve a general and uniform response to most tissues and organs with possible exceptions for coronary and cerebral blood flow Recent studies using the same technique as applied presently on blood flow distribution during arousal from hibernation in mammals have demonstrated a striking parallel to the present findings in regards to a segmentally oriented change in sympathetic vasomotor outflow (Johansen 1961 Bullard and Funkhouser 1962) Both these publications demonstrate a conspicuous increase in blood flow to organs anterior to the heart while the posterior portion of the body remains vasoconstricted

It seems of interest that a 3 to 5-fold increase in flow to tissues like the ventricular myocardium and central nervous tissue will increase or maintain a relatively unchanged oxygen availability to these tissues during the asphyxia period investigated Specialized and vital tissues may possess a faculty of regulating their blood flow also by more local or intrinsic mechanisms The finding that the adrenals showed a conspicuous increase in activity while most other visceral organs were markedly reduced in activity serves to emphasize this point The significance of an increased blood flow to the adrenals *during submersion asphyxia is hard to evalute on the basis of present information* but invites further studies

The question naturally emerges whether the response obtained on naturally diving animals during submersion asphyxia is a specialized phenomenon or a more general defense mechanism to asphyxia in all higher vertebrates In spite of an overwhelming literature on circulation during asphyxia in mammals few generalizations are justified at present Applications of anesthesia and general experimental conditions differ to the extent that such a comparison is very restricted It seems relatively established however that renal blood flow is markedly decreased during asphyxia in mammals generally (Murray and Young 1963

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it actually decreases to the heart and to several tissues and organs cranial to the level of the heart. This finding seems at first hand to be exceptional as the general concepts about an increased sympathetic discharge in the vasomotor nerves involve a general and uniform response to most tissues and organs with possible exceptions for coronary and cerebral blood flow. Recent studies using the same technique as applied presently on blood flow distribution during arousal from hibernation in mammals have demonstrated a striking parallel to the present findings in regards to a segmentally oriented change in sympathetic vasomotor outflow (Johansen 1961, Bullard and Funkhouser 1962). Both these publications demonstrate a conspicuous increase in blood flow to organs anterior to the heart while the posterior portion of the body remains vasoconstricted.

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The question naturally emerges whether the response obtained on naturally diving animals during submersion asphyxia is a specialized phenomenon or a more general defense mechanism to asphyxia in all higher vertebrates. In spite of an overwhelming literature on circulation during asphyxia in mammals few generalizations are justified at present. Applications of anesthesia and general experimental conditions differ to the extent that such a comparison is very restricted. It seems relatively established, however, that renal blood flow is markedly decreased during asphyxia in mammals generally (Murray and Young 1963).

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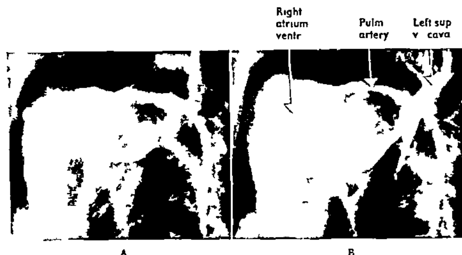


Fig 1 Angiocardiography depicting inflow veins right heart and pulmonary artery before (a) and during (b) submersion

Note the delayed emptying of contrast and the reflux into adjoining veins during submersion. Note also the dimensional changes of the pulmonary arteries and the right side of the heart The reduction in pulmonary artery diameter during submersion was most conspicuous in its central part Exposures done in mid-diastole

vascular function in this situation (Eliassen 1960 Johansen and Aakhus 1963) The conspicuous bradycardia and alleged peripheral vasoconstriction during submersion are highly suggestive of other important circulatory changes

Angiocardiography with film changer technique represents a useful tool for evaluation of the central circulation The technique has hitherto not been used in the study of phenomena during submersion of diving animals The present study takes advantage of this technique supported by simultaneous arterial pressure measurements

Materials and methods

Domestic ducks were used for the experiments No anesthesia was used except for the local application of xylocain at the sites of cannulation The contrast medium Isopaque Nycomed 60 was injected through a catheter in the right or left superior caval vein or directly into the right atrium through a polyethylene catheter introduced in the right brachial vein The position of the catheter was checked by fluoroscopic control. The volume of contrast medium ranged between 4—6 ml and was injected with an automatic pressure syringe

The roentgen equipment consisted of an Elema Schonander film changer The rate of exposure varied up to six frames per sec in the pre-submerged animals against two frames per sec in the submerged condition The exposures were done at 62 kV and 200 mA exposure time 0 03 sec film focus distance 90 cm and focus size 1.2 × 1.2 mm

Arterial pressure was continually recorded through a catheter in the aorta with the aid of a Statham pressure transducer a Sanborn carrier preamplifier and a 4-channel Sanborn recorder Timing and duration of contr[illegible] ction as well as the film exposures were recorded together with th[illegible] bo n recorder

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The animals were fastened to a board in normal prone sometimes slightly oblique position Diving was simulated by manually submerging their head in a water container The periods of submersion lasted up to 3 min

Results

The contrast injections outlined the inflow veins to the right atrium clearly It was invariably observed that the contrast density was markedly greater in the inflow veins during the submerged condition A delayed emptying of contrast from the inflow veins and variable degrees of reflux into adjoining veins were similarly commonly observed during submersion Fig 1 illustrates this point showing filling of the left superior vena cava in the pre submerged (A) and submerged (B) condition Table IA and IB represent a condensation of some of the functional characteristics obtained from the radiological data Data referring to pre submerged and submerged conditions are grouped separately The letters on the top of each column indicate the different ducks used in the experiments

Timing of the cardiac cycle

The development of bradycardia during submersion is gradual and does not reach a maximum until about 40—50 sec after start of submersion (Johansen and Aakhus 1963) The roentgen exposures were as a rule made after the conspicuous bradycardia had been established

In the normally breathing animal prior to submersion the circulation time from start of injection to the appearance of contrast in the pulmonary artery was about 1 sec representing 3 heart beats (in one instance 5 heart beats) compared to a range from 2—4 sec and 1 heart beat in the submerged condition In exp B (submerged condition) the bradycardia was only moderate requiring 3 heart beats to bring the contrast to the pulmonary artery The pulmonary capillary phase became discernible after 2 sec in the animals breathing normally compared to 2—8 sec in the submerged condition The duration of the capillary phase evaluated from the roentgen frames was about 2 sec in the normally breathing animals against more than 14 sec in the submerged animals A similar difference in circulation time was clearly detectable after the appearance of contrast in the pulmonary vein and left atrium This was effected in about 2 sec in the normally breathing animals compared to 6—12 sec in the submerged animals Again a smaller number of heart beats sufficed to propel the contrast in the submerged animals 4—5 beats against 5—9 beats in the pre-submerged condition One heart beat after the contrast was discernible in the left atrium the left ventricle was usually well defined in the submerged animal At approximately the same time the larger systemic arteries the aorta and the brachio-cephalic arteries became distinctly visible It took about 3 sec and 7—9 heart beats to bring the contrast to the aorta in the normally breathing animals compared to 7—14 sec and 5—6 heart beats in the submerged animals

Dimensional changes

A rather conspicuous difference in the dimensions of the heart chambers and greater vessels became apparent when comparing the animals prior to and during submersion The total size of the heart was always increased during submersion in corresponding phases of the cardiac cycle (Fig 2) In one exp (C) the projected area of the heart

A B

Fig 2 Anterio-posterior view of the chest A pre submerged B submerged The heart size is considerably increased and the projected lung area reduced during diving



Fig 3 Angiocardiography demonstrating dimensional changes of the pulmonary artery and right heart before (a) and during (b) submersion Exposures done in systole

The animals were fastened to a board in normal prone sometimes slightly oblique position Diving was simulated by manually submerging their head in a water container The periods of submersion lasted up to 3 min

Results

The contrast injections outlined the inflow veins to the right atrium clearly It was invariably observed that the contrast density was markedly greater in the inflow veins during the submerged condition A delayed emptying of contrast from the inflow veins and variable degrees of reflux into adjoining veins were similarly commonly observed during submersion Fig 1 illustrates this point showing filling of the left superior vena cava in the pre-submerged (A) and submerged (B) condition Table IA and IB represent a condensation of some of the functional characteristics obtained from the radiological data Data referring to pre submerged and submerged conditions are grouped separately The letters on the top of each column indicate the different ducks used in the experiments

Timing of the cardiac cycle

The development of bradycardia during submersion is gradual and does not reach a maximum until about 40—50 sec after start of submersion (Johansen and Aakhus 1963) The roentgen exposures were as a rule made after the conspicuous bradycardia had been established

In the normally breathing animal prior to submersion the circulation time from start of injection to the appearance of contrast in the pulmonary artery was about 1 sec representing 3 heart beats (in one instance 5 heart beats) compared to a range from 2—4 sec and 1 heart beat in the submerged condition In exp B (submerged condition) the bradycardia was only moderate requiring 3 heart beats to bring the contrast to the pulmonary artery The pulmonary capillary phase became discernible after 2 sec in the animals breathing normally compared to 2—8 sec in the submerged condition The duration of the capillary phase evaluated from the roentgen frames was about 2 sec in the normally breathing animals against more than 14 sec in the submerged animals A similar difference in circulation time was clearly detectable after the appearance of contrast in the pulmonary vein and left atrium This was effected in about 2 sec in the normally breathing animals compared to 6—12 sec in the submerged animals Again a smaller number of heart beats sufficed to propel the contrast in the submerged animals 4—5 beats against 5—9 beats in the pre submerged condition One heart beat after the contrast was discernible in the l ft atrium the left ventricle was usually well defined in the submerged animal At approximately the same time the larger systemic arteries the aorta and the brachio-cephalic arteries became distinctly visibl It took about 3 sec and 7—) heart beats to bring the contrast to the aorta in the normally breathing animals compared to 7—14 sec and 5—6 heart beats in the submerged animals

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A B

Fig 2 Anterio-posterior view of the chest A pre submerged B submerged The heart size is considerably increased and the projected lung area reduced during diving



Fig 3 Angiocardiography demonstrating dimensional changes of the pulmonary artery and right heart before (a) and during (b) submersion Exposures done in systole

TABLE I Part A demonstrates heart rate and blood pressure values correlated with circulation systemic vascular beds Part B shows the dimensional changes of the pulmonary the different ducks used in the experiments Duck D was submerged twice

			Pre-submerged	
			A	B
A Heart rate			150	180
Systemic arterial blood pressure	mm Hg	Systolic	200	150
		Diastolic	140	60
Pulmonary artery	First appearance	Seconds	1	1
		Beats	3	3
Pulmonary capillary phase	First appearance	Seconds	2	2
		Beats	5	3
		Duration seconds	2	4
Pulmonary vein and left atrium	First appearance	Seconds	PV 2 LA 3	2
		Beats	PV 5 LA 7	6
Left ventricle	First appearance	Seconds	3	3
		Beats	7	8
Aorta and brachio-cephalic arteries	First appearance	Seconds	3	3
		Beats	7	8
B Dimensional changes (mm)				
Pulmonary artery	Systolic	Central	13 5	15 2
		Peripheral	10 5	9 4
	Diastolic	Central Middle	8 2	
		Peripheral	5 9	

contour was measured by planimetry giving approximately 20 cm² prior to submersion against 32 cm² during submersion The difference was even more marked when the difference in size of the right heart (right atrium and right ventricle together) was measured Fig 1 This revealed 8 7 cm² prior to submersion compared to 20 1 cm² during submersion The left ventricle displayed a more variegated picture however not obscuring a tendency to an increased volume and a larger systolic residue during submersion On one animal calculations were made of the dimensional changes of the area projected by the thoracic cavity There was an approximate 10 reduction in this area during submersion The simultaneous increase in heart size contributes further to a reduced thoracic volume during submersion

Among the larger vessels the pulmonary arteries displayed the most notable dimensional changes The vessel diameter was measured on the left pulmonary artery shortly after the bifurcation of the pulmonary trunk This segment of the vessel would presum

times from the inject on site in the systemic veins to various segments of the pulmonary and arteries during pre submerged and submerged The letters on top of each column indicate

Pre submerged			Submerged					
C	D	E	A	B	C	D	D	E
180	260	200	24	50	20	35	30	32
200	160	200 175	190	165	200	185	175	215
150	175	150 75	75 100	60	150	100	100 110	140
1	1	0 75	2 5	3	4	2	2	3 5
3	5	3	1	3	2	1	1	
2	2	1 25	2	3	8	2	6	3 5
	9	4	5	3	3	1	3	
<2	2	<2	>8.5	>14	>14	>14	>14	>14 5
2	2	2 5		6		8	12	8
5	9	6		4		4	5	
	2	3 25		6		10	14	9
9	9	8		4		5	6	
3	2	3 5		7		12	14	10 5
9	9	8		5		5	6	
10 5	11 9	11 7	10 0		5 2	10 0	11 6	7 0
8 0	9 4	7 7	10 0		5 5	8 3	9 0	7 0
		7 0	4 7	6 4		3 8	4 9	3 9
			3 6	3 2			3 2	
		6 7	6 4	7 5		7 2	7 6	4 7

ably be spaced freely in the thoracic cavity Dimensions were also measured at a more peripheral site where the artery was embedded in the pulmonary parenchyma The dimensional changes were timed to specific points in the cardiac cycle evaluated from the arterial pressure tracing recorded simultaneously The composite results are available from Table IB The numbers designating the diastolic dimensions are measured in the end-diastolic phase The essence of the dimensional changes consists first of all in far smaller vessel diameters during submersion (Figs 1 and 3) In animal C the heart rate changed from 180 to 20 beats per min and the systemic arterial blood pressure stayed unchanged going from pre submerged to submerged condition The maximum systolic central diameter was 10 5 mm before compared to 5 2 mm during submersion The peripheral diameter changes were not nearly as striking being for animal C 8 0 mm systolic value prior to submersion compared to 5 5 mm during submersion (Table I B) The dimensional changes *in the diastolic phase were in con*

sonance with the systolic changes (Table 1 B) The diameter reduction during submersion was by far most distinct in the segment of the artery confined to the free thoracic space (Figs 1 and 3) This feature was so dominating that the paradoxical situation of a pulmonary artery with smaller central than peripheral diameter was observed in many animals (Table I B Fig 1 B) Another common feature of the pulmonary artery during submersion was the presence in diastole of a particularly narrow segment about midway between the base of the artery and the peripheral measuring site

Any dimensional changes in the larger systemic arteries the aorta or the brachiocephalic arteries were hardly detectable comparing the pre submerged and submerged conditions

Discussion

The present finding of a marked tendency of venous reflux during contrast injection in the submerged condition is suggestive of an increased central venous pressure This is in agreement with the results of previous measurements of central venous pressure Johansen and Aakhus (1963) recorded a pressure increase in the central veins amounting to 15—20 mm Hg during submersion The greater contrast density and the delayed emptying observed presently in the larger veins during submersion imply a reduction in the volume flow through these vessels An increased resistance to cardiac filling caused by an increased intra thoracic pressure may also be reflected in the venous reflux and the delayed emptying of contrast medium from the central veins

The most consistent finding in the radiological data was the delayed passage of the contrast medium through the pulmonary vascular bed during submersion There was a doubling of the time required to bring the contrast from the injection site to the pulmonary artery during submersion Similarly there was more than a fivefold increase in time for the contrast passage through the entire pulmonary vascular bed This finding is in close agreement with earlier observations (Johansen and Aakhus 1963) utilizing a modified thermodilution technique in the evaluation of pulmonary circulation time during submersion It should be emphasized that the degree of bradycardia developed during submersion is a decisive factor for the degree of conformity between individual experiments The same reservation should be made when comparing the results of different investigators The duration of the pulmonary capillary phase evaluated from the roentgen exposures was more than seven times longer during submersion

The prolongation in circulation times observed during submersion is not directly expressive of parallel changes in volume flow However taking into account the rather profound dimensional changes recorded in the present study the volume flow must be radically reduced A reduction in heart frequency to 10—20 % of initial values during submersion would require a five to tenfold increase in stroke volume in order to maintain an unchanged cardiac output Such a compensation seems highly unlikely and a decreased cardiac output during submersion seems probable Earlier data (Johansen and Aakhus 1963) showed no change in systolic ejection time and insignificant increases in intraventricular pressure concurrent with an unchanged arterial pressure during submersion On these grounds it may be deduced that no major changes in stroke volume can have taken place

One causative factor in the probable increased resistance to flow in the pulmonary bed during submersion seems to be an increased intrathoracic pressure Such an

increase has been measured directly (Johansen and Aakhus 1963) and additional support is offered by the reduction in the area projected by the thoracic cavity as observed presently Eliassen (1960) has similarly measured an increase in pulmonary arterial pressure during submersion Another possible influence may be a direct effect of the low alveolar oxygen tension on the vascular smooth muscle of the pulmonary vessels (Nisell 1948 Duke 1957)

The reduction in caliber of the pulmonary vessels must influence the blood torage ability of the pulmonary vascular bed A reduction in pulmonary blood volume during submersion seems to be a rational adjustment to the prolonged period of asphyxia It is of interest to note in this regard that the intrathoracic systemic veins display no or rather insignificant changes in caliber while the larger pulmonary vessels may be reduced to half the size seen prior to submersion This indicates that the increase in intrathoracic pressure is balanced by an increase in central systemic venous pressure giving no changes in transmural pressure whereas the pulmonary vessels must be subjected to a decrease in transmural pressure

The consistent finding of an increased size of the heart particularly of the right side during submersion brings into focus the highly debated question of factors other than the end diastolic distention as determinants of the ventricular discharge Several workers have recently stressed the modifying effect on ventricular energy release mediated through the cardiac nerves and by humoral agents (Rushmer 1955 Mitchell Linden and Sarnoff 1960) The conspicuous increase of the heart size in diving animals as a normal physiological response provides a striking example of regulating factors capable of over ruling the end-diastolic distention in adjusting the stroke output

A study of these regulating factors in the naturally diving animals may represent a fruitful object for further research

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The Effect of Exercise on the Vascular Bed of Skeletal Muscle

By

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Abstract

Kjellmer I *The effect of exercise on the vascular bed of skeletal muscle* Acta physiol scand 1964 62 18—30 — A plethysmographic method is described which has been used for continuous recording of volumetric changes in the isolated calf muscles of cats. Venous outflow, arterial and venous pressures were measured simultaneously. Steady state exercise was mimicked by stimulation of the motor nerve. Changes occurring in the resistance vessels, in the capacitance vessels and in the capillary filtration coefficients were recorded. Exercise provoked a dilatation of the resistance vessels, an increase of the capillary filtration coefficient (dilatation of the precapillary sphincters) and a distension of the capacitance vessels. The degree of dilatation in all three vessel sections was related to the severity of exercise. It was shown that the increase in regional blood volume (dilatation of the capacitance vessels) is caused mainly by an increased pressure head reaching the capacitance vessels and not by an active venous dilatation. During exercise there was a net outward capillary filtration due to an elevated capillary hydrostatic pressure and augmented by an increased capillary surface area available for blood flow. The elevation of capillary pressure was caused by a dilatation which affected the precapillary resistance vessels relatively more than the postcapillary [illegible]

It is well known that muscular exercise causes a local dilatation of the vessels and an increased flow of blood to the active muscles. In his classical study Krogh (19[illegible]) showed that the number of blood-containing capillaries per unit of cross-sectional area of a given muscle is much larger in active than in resting tissue.

During exercise there is thus a dilatation of the resistance vessels simultaneous with an opening up of precapillary sphincters with consequent spread of the flow over an increased number of capillaries. The effect of exercise on the third important vascular compartment, the capacitance vessels, mainly the venules and veins, appears to have received little attention.

This paper is concerned with the largely simultaneous reactions of the resistance vessels, the capillaries and the capacitance vessels of the skeletal muscle to graded exercise and estimation of the variation of mean capillary pressure and of net filtration exchange across the capillary membrane.

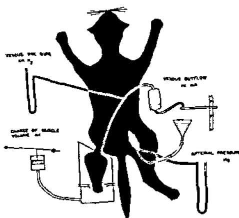


Fig 1 Schematic drawing of the experimental set up

Methods

The method used was principally that utilized by Mellander (1960) but adapted for the vascular bed of the calf of the cat

Thirty-eight cats of both sexes weighing between 2 0 and 5 5 kg were used The anesthesia was induced with ether and either chloralose (70 mg/kg) chloralose (50 mg/kg) plus urethane (100 mg/kg) or nembutal (30 mg/kg) was given intravenously Heparin about 5 mg/kg was administered to prevent clotting A free airway was secured by means of a tracheal cannula Rectal temperature was maintained at 38 with a heating pad regulated by a thermostat

Operation The right paw was exarticulated at the ankle joint. A ligature was tied around the achilles tendon and another around the tendons of the extensor muscles of the toes These ligatures were then tied together over the bone and the calf muscles thereby stretched The exposed bone was covered with the skin from the paw and a plastic sheet The skin around the entire circumference of the limb was divided as high up the thigh as possible and dissected free from subcutaneous tissue down to the level of the knee joint The thigh muscles were then severed just above the knee joint by thermocautery The femur was drilled and the bone marrow cavity plugged with cotton soaked in vaseline The sciatic nerve was divided about 5 cm above the knee joint The large saphenous vein was divided between ligatures as was the small saphenous vein and the saphenous nerve with the accompanying artery The popliteal artery and vein were freed and all small branches were ligated with the result that these two vessels were the only ones running to and from the calf muscles

The calf was placed in a plethysmograph and fixed firmly in position by means of two screw clamps gripping the ankle The plethysmograph was sealed with the skin that had previously covered the thigh and filled with water maintained at 38 by a thermostatically regulated heating lamp The water filled the funnel of the plethysmograph to a level 6—8 cm above the centre of the tissue mass

In nine of the experiments, in which a constant blood flow was desired the flow from the right femoral artery was conducted through a Sigmamotor pump Model TM 11 to the popliteal artery The temperature of the perfusing blood was registered with an electrical thermometer with thermocouples in the blood stream and kept at 36—38 by heating lamps

The peripheral end of the severed sciatic nerve was placed on bipolar silver electrodes and stimulated with square wave pulses from a Grass Stimulator Model S4 CR The stimulation characteristics were 1—3 V 0 1 msec and 0 5—4 impulses per second The stimulation intensity chosen was such that judging from the mechanical artifact produced by each single twitch on the volume record and from the blood flow *response all muscle fibres* were activated in each experiment The intensities however were far too low to evoke any response of the vasoconstrictor fibres in the sciatic nerve This was checked in the following way In five experiments where the sciatic nerve was not cut the ipsilateral lumbar sympathetic chain was isolated At the end of the experiment gallamine triethiodide (Flaxedil®) was given in a dose large enough

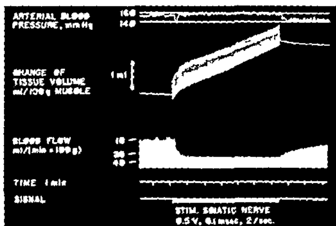


Fig 2 Cat 4 0 kg Effects of muscle contractions on blood pressure tissue volume and blood flow In this figure (as in Fig 4 5 and 7) the volume artifact during exercise is due mainly to a small displacement of the tissue in relation to the plethysmograph during each contraction The volume artifact has been retraced in these figures from the original recording

to block the neuro-muscular junction Stimulation of the sciatic nerve using the same characteristics as before now produced no changes in blood flow through the muscles while stimulation of the sympathetic chain showed that the vasoconstrictor fibres could still be activated Even when the stimulation intensities applied to the sciatic nerve after administration of Flaxedil were markedly increased (to above 30 V) hardly any vasoconstrictor response could be elicited

After each experiment the muscle mass enclosed in the plethysmograph was carefully dissected free from surrounding tissues (skin and bone) and weighed The mean weight of the calf muscles in 27 experiments was 17 4 g/kg b w (S D = 1 5) In the remaining experiments the muscle weight was estimated from the body weight

Recording The volumetric changes in the plethysmograph were measured with an air filled piston recorder usually with a deflexion of 50—60 mm per ml The arterial inflow pressure was recorded with a mercury manometer connected with the opposite femoral artery In the constant perfusion experiments the pump pressure was measured with a mercury manometer in the perfusion tubing immediately distal to the pump

The venous outflow was conducted first from the cannulated popliteal vein into a drop chamber containing silicone oil and then back to the opposite femoral vein via a collecting funnel The blood flow was recorded by means of a photocell device attached to the drop chamber and operating an ordinate writer The product of the blood flow and the height of the ordinate was constant and the height of the ordinates therefore inversely related to the magnitude of the blood flow

The venous pressure in the tubing running to the drop chamber was measured with a water manometer The zero level of this manometer was placed at the level of the popliteal vein in the preparation (1—2 cm above the right atrial level) Since blood flow was recorded with an open system the venous outflow pressure could be set at any desired level by adjustment of the level of the outlet from the drop chamber

All recordings were made on a slowly moving smoked paper on a kymograph drum The set up is shown schematically in Fig 1

Procedure When all preparatory operations had been performed blood flow was allowed to become stable and venous outflow pressure was adjusted to achieve an isovolumetric state usually at a level of 8—12 cm H_2O It should be noted that this corresponds to a venous pressure not more than 3—4 cm H_2O above the water pressure in the plethysmograph To enable determination of the capillary filtration coefficient (CFC) during rest and exercise venous outflow pressure was repeatedly raised for a few minutes (1—3) at a time to a level 10—20 cm H_2O above the initial level This procedure resulted in a diphasic change in volume first a phase of rapid venous filling and then a phase of slower outward filtration The elevation of venous pressure was continued until the slope of the slow phase was clearly evident

Calculations The capillary filtration coefficient (CFC) was calculated from the volume curve by measuring the change in net capillary fluid exchange during the brief periods of raised venous

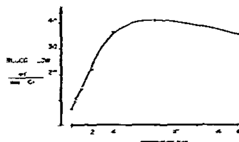


Fig 3 Ca 3.6 kg Blood flow in relation to work load, expressed as contractions sec.

pressure. Calculations of CFC requires knowledge of the change of mean capillary hydrostatic pressure on elevation of the venous pressure. This change in pressure was estimated by assuming that 80 per cent of the change in venous pressure was transmitted to the capillaries. In experiments with a constant perfusion the corresponding figure was assumed to be 1.0 per cent. The basis of these assumptions as well as estimations of the error in determining CFC are given below (Comments on method).

Results

All values are expressed per 100 g muscle weight.

Blood flow During a period of exercise consisting of a series of induced muscular contractions the blood flow increased during the first one or two minutes to reach a level at which it remained fairly constant as long as the exercise was continued (Fig. 2. Using supramaximal stimuli the exercise hyperemia was graded by changing the frequency of the single twitches until maximum vasodilation was reached. Fig. 3 shows the relation between the blood flow and frequency of the contractions in a representative experiment. Only those parts of the individual experiments, where the muscular contractions were induced at the rate of at most 4 per second were used for deductions since higher frequencies often appeared to cause a considerable mechanical hindrance to blood flow. In most cases 4 contractions per second induced a vasodilatation close to maximum (cf. frequency response curve in Fig. 3).

The blood flow resistance expressed as (mm Hg / min / 100 g ml) was about 29 peripheral resistance units (PRU) in the resting muscle with sympathectomized vessels and decreased to about 3 units during the most severe exercise (Table I.

Tissue volume The changes in tissue volume during exercise are included in Fig. 2. The curve was invariably diphasic: the first rapid phase coincided with the initial acceleration of the blood flow, the second, less rapid phase with the level at which the blood flow remained steady.

The initial rapid increase in volume was presumably due to an increase of the regional blood volume the degree of which varied with the severity of exercise. Table I.

The second phase consisted of a less rapid continuous increase of volume which was not due to any further relaxation of the capacitance vessels (see discussion) but was attributed to a net movement of fluid into the tissue. To differentiate between filtration and diffusion the same exercise was performed at different perfusion pressures in five cats. Fig. 4 gives a typical example. Reduction of the perfusion pressure abolished both the first and the second phase of the volume response. The abolition of the second phase can be accounted for provided this change is normally caused by filtration but not if the change normally is caused by diffusion (see discussion.

TABLE I

Exercise twitches/sec	Number of observations	PRU (mm Hg × min × 100 g/ml)	Increase of regional blood volume (ml/100 g)	Rate of outward filtration (ml/min × 100 g)	Calculated increase in capillary pressure (mm Hg)
0	86	17.0 8.8–33.3	0	0	0
0.5	7	7.5 4.2–13.7	0.35 0.16–0.61	0.11 0.03–0.20	4.7 1.5–9.0
1	36	5.2 2.0–12.9	0.55 0.20–1.14	0.26 0.08–0.50	9.9 3.5–27.0
2	19	4.1 2.4–7.7	0.73 0.21–1.12	0.48 0.12–0.76	13.8 5.5–31.0
3–4	7	3.0 1.6–5.4	0.82 0.43–1.24	0.75 0.36–1.00	17.3 10.0–25.0

For each parameter mean value and range is given

The outward filtration during exercise seems to be ascribable to increased capillary pressure whose effect is enhanced by a larger capillary filtration coefficient (CFC) which means that for a given increase of capillary pressure more filtrate is formed per minute Fig 5 gives an example of the CFC during rest during exercise and after exercise In this particular experiment the CFC rose from 0.012 ml/(min × mm Hg × × *100 g) during rest to 0.036 ml/(min × mm Hg × 100 g) during steady state exercise* while blood flow increased from 8 ml/(min × 100 g) to 40 ml/(min × 100 g) When exercise was stopped the CFC gradually diminished roughly in proportion to the diminishing blood flow The relation between CFC and blood flow resistance expressed as peripheral resistance units PRU — (mm Hg × min × 100 g)/ml — is shown in Fig 6 (solid symbols) Changes in PRU were brought about by induced exercise of varying severity As can be seen from this figure CFC increased up to three fold together with a decrease in blood flow resistance from the control range of about 20 units to about 3 units e g while resistance to blood flow dropped to about one sixth of the resting value resistance to filtration exchange decreased to about one third Although the scatter of CFC values was wide in each single experiment the CFC increased as soon as PRU decreased Thus the scatter is caused mainly by large differences between different experiments

With knowledge of the value for net capillary outward filtration during exercise and the CFC value it is possible to calculate the change of mean capillary hydrostatic pressure by dividing the former value in ml/(min × 100 g) by the latter in ml/(min × 100 g × mm Hg) The elevation of capillary pressure during exercise is given in Table I together with the filtration values As will be discussed below the change in capillary pressure during exercise is probably somewhat overestimated while the changes of CFC are correspondingly underestimated

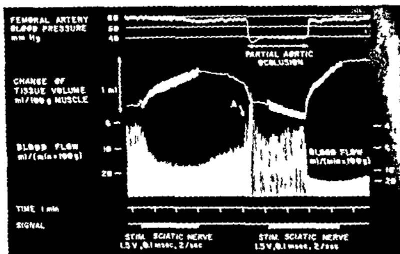


Fig 4 Cat 3 0 kg Effect of reducing perfusion pressure on the response of the tissue volume to exercise At point A the rising rate for the ordinate writer was reduced (see scale on right hand side on Fig)

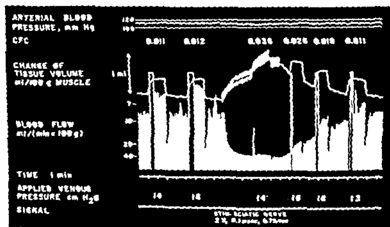


Fig 5 Cat 2 4 kg Effects of muscle contractions on blood pressure capillary filtration coefficient (CFC) tissue volume and blood flow

Constant perfusion experiments Nine cats in which the blood flow to the calf was delivered from a constant perfusion pump were included in the study in order to secure a more accurate estimation of CFC (see below Comments on method) secondly to provide a further possibility of differentiating filtration from diffusion and thirdly to ascertain whether the initial increase in regional blood volume during exercise is due to an active dilatation of the capacitance vessels or to distension caused by an increased pressure head

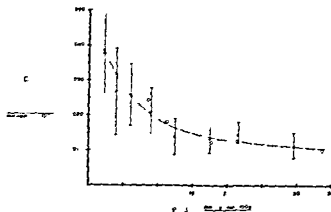


Fig. 6. Relation between the capillary filtration coefficient (CFC) and blood flow resistance expressed as peripheral resistance units (PRU).

Hollow symbols — experiments with perfusion pump; solid symbols — experiments without pump. ± 1 standard deviation is indicated. Line drawn by inspection. Values classed according to PRU values. 92 determinations during rest and 108 during exercise without constant perfusion. 33 during rest and 43 during exercise with perfusion pump.

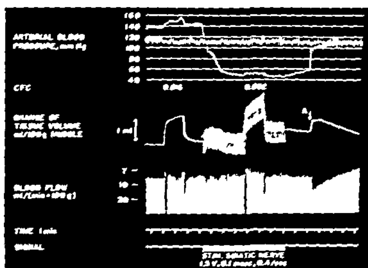


Fig. 7. Cat 3.6 kg. Effects of muscle contractions on perfusion pressure, capillary filtration coefficient, CFC, tissue volume and blood flow in an experiment where a constant perfusion pump was used.

The starting level of the perfusion pressure exceeds the systemic blood pressure by about 20 mm Hg. At point A the muscles were perfused directly from the femoral artery.

A representative experiment is shown in Fig. 7. When exercise was induced the reduction of blood flow resistance in the calf muscles was reflected by a diminishing perfusion pressure which persisted at a fairly stable low level during the whole period of exercise. The tissue volume first increased somewhat, but as the perfusion pressure

fell the muscle volume returned almost to the initial level and despite continued muscle contractions the tissue volume remained practically unchanged

CFC values were noted repeatedly before during and after exercise and varied inversely with blood flow resistance Fig 7 shows the recording of one CFC during rest and one during exerci e Note that when the venous pressure was raised 10 mm Hg the constant perfusion pump automatically elevated the arterial pressure so that the per fusion pressure was essentially unchanged During rest this was always the case while during vasodilatation caused by exercise the increase of arterial pressure was usually 70 to 80 per cent of the applied change of venous pressure The relation between CFC and PRU in these experiments is given in Fig 6 (hollow symbols)

Comments on method for CFC determination

To estimate CFC the venous pressure is abruptly raised by a known amount (in this study usual ly about 15 cm H O) The ensuing increase in volume of the limb studied is diphas c first a rapid phase due to distension of the capacitance vessels and then a slower but continuous phase of outward filtration These tw o phases have been discussed by e g Pappenhe mer and Soto-Rivera (1948) and Mellander (1960)

*Calculation of the CFC requires knowledge of the amount by which the mean capillary pres*sure is increased by the elevation of venous pressure It has been shown that the ratio between precapillary and postcap llary resistances (r r) determines what fraction of the increment of venous p essure reaches the capillary level (cf Bayliss and Starling 1894) The present ex perim nts do not allow d rect determination of this ratio w th accuracy Instead a certain ratio has to be estimated If for instance the arterial p essure is 100 mm Hg the mean cap l lary pressure is 20 mm Hg and the venous pressure is cl se to 0 the r r ratio is 4 1 which means that 80 per cent of the change in venous pressure is transmitted to the capillary level All CFC values reported here (except those recorded in the constant perfusion experiments) were calculated us ng this figure of 80 per cent.

The maximum error in this assumption was estimated by taking into account the total range of arterial and venous pressures in these experiments The mean arterial pressu e ranged be tween 80 and 160 mm Hg while the venous pressure was kept fairly constant at 5—10 mm Hg Maximum range for the r r rat os at ma ntained isovolumetric state was then 14 1 — 4 1 For comparison the corresponding values at a mean capillary pressure assumed to be 30 mm Hg are 6 5 1 and 2 1 and at a mean capillary pressure of 15 mm Hg the values e 29 1 a d 6 5 1 At first sight th s seems to be an extremely wide range and the redu tion factor of 80 per cent thus an extremely unreliable assumption but if the reduction facto is actually calculated it will be found to vary between 67 and 97 p r cent when the r r rat o varies by as much as from 2 1 to 29 1 The use of a reduction f ctor of 80 per cent then means that the estimation of CFC has an error of less than 20 per cent.

Dur g exercise there is an ncrease of the mean capillary pressure by about 10 mm Hg (Table 1) To demon trate *the effects of such a cha ge on the error in the CFC-determination* one can compare the values given above at capillary pressures of 20 and 30 mm Hg Calculating the CFCs during rest and exercise with the same reduction facto would thus mean under estimation of the increase of CFC by 15—20 per cent a figure contrasting sharply with the observed changes of 200—300 per cent

Any underestimation of the CFC during exe cise leads to overest mation of the change of mean capillary pressure by at most 15—20 per cent of the observed change

To estimate the error n the CFC value in a more direct way nine experiments were per formed using the constant perfusion technique described abo e The flow ate of the pump was set at a value clos to the resting blood flow When th venous pressu e was increased abruptly the pump increased its pressure by about the same amount s nce w th a constant blood flo v and a presumably fairly constant blood flow res stance the perfus on press ire should be essentially unchanged In these instances the total increment of venous pressure is presumably transmitted to the capillary level since the p essure curve for the whole vascular bed in the calf changed simultaneously by an amount equal to the elevation of the venous pressure

When 10 mm Hg was appl ed during rest the pump pressure rose by the same amount, but during exercise the pump pressure usually rose only 7—8 mm Hg (Fig 7)

There is at least one reason why the pump pressure should rise less than the venous pressure when the venous pressure is raised the distending pressure is increased and owing to the distensibility of the resistance vessels the blood flow resistance is decreased. On the other hand, the higher distending pressure tends to excite the myogenous automaticity of the smooth muscles in the vessel walls (Bayliss 1902 Folkow 1962) which leads to a secondary rise in the blood flow resistance. During rest these two opposing tendencies obviously counterbalance one another while during vasodilatation produced by exercise the tendency towards decreased flow resistance dominates This is in accord with the findings of Folkow and Lofving (1956) and Roddie and Shepherd (1957) who showed that the distensibility of the resistance vessels is highest when vascular tone is low

It thus seems safe to assume that during rest 100 per cent of the applied venous pressure is transmitted to the capillaries with the constant perfusion technique This assumption will also hold roughly for the situation during exercise, since the major part of the 20—30 per cent reduction occurs at the precapillary level. However also during this type of experiment the changes of CFC during exercise are slightly underestimated.

To compare the results obtained in the two series of experiments (with and without constant perfusion) the CFC values were plotted against the peripheral blood flow resistance The values were divided in classes according to the PRU values and the mean for each class was calculated The solid symbols in Fig 6 gives the relation without and the hollow symbols with constant perfusion pump The values compare closely in the two series but it is obvious that the PRU values noted during the severest exercise were about twice as high in the series with the pump as in the one without. This was expected since when the rate of blood flow is kept constant, the pump pressure falls to around 30—35 mm Hg at maximum vasodilatation which means that the distending pressure is reduced to a very low value with an ensuing less efficient dilatation (cf. Folkow and Lofving 1956) PRU values of 5 to 10 (mm Hg × min × 100 g)/ml seem to be the limit of vasodilatation at such low arterial pressures Furthermore it should be realized that at any given vascular tone not only should the resistance be higher but also the CFC values probably lower in the constant perfusion experiments due to passive elastic recoil occurring in response to the decreased transmural pressure in these experiments When these facts are taken into account the two series of values in Fig 6 compare so closely that it seems justified to draw the conclusion that CFC values obtained without the perfusion pump are as reliable as those with the pump technique.

Discussion

The dilatation of the resistance vessels during exercise has been extensively studied for about one hundred years The relation between muscular exercise and hyperemia — here depicted in Fig 3 — was studied among others by Kramer Obal and Quensel (1938) who found a direct relationship between increasing strength of contraction and hyperemia up to a point where the hyperemia was prevented from increasing any further by the fact that maximal vasodilatation was reached and because of the increasing interference created by mechanical obstruction of the vessels Barcroft and Dornhorst (1949) showed that strong rhythmic contractions of the human calf impeded blood flow markedly during the actual contraction Black (1959) measured blood flow through the calf of humans walking and running the same distance at varying speeds and found that blood flow increased linearly with pace rate up to a certain point. When the pace rate exceeded this point peak flow after exercise increased only little while the amount of excess blood flowing after exercise increased. This is a parallel to the findings reported here that blood flow is directly related to the severity of exercise expressed in terms of number of contractions per unit of time (Fig 3) In these experiments, too a certain point was reached — usually about 4 contractions per second — after which further increase of the severity of exercise did not cause any proportional change of blood flow and where blood flow increased markedly as soon as exercise was stopped All experiments that showed signs of mechanical obstruction to flow were excluded.

Exercise was accompanied by a dilatation of the resistance vessels and of the capacitance vessels (Fig 2 and 5) the dilatation varying with the severity of the exercise (Table I) The dilatation of the capacitance vessels was partly an artifact caused by an elevated venous pressure due to the increased pressure drop over the drop chamber and tubing during the hyperemia This elevation of venous pressure was about 2 cm H_2O at the highest blood flows Since the distensibility of the veins in this preparation was about 0 06 ml per cm H O and 100 g this rise of venous pressure could account for up to 20 per cent of the widening of the capacitance vessels The remaining part is probably due largely to an increased pressure head reaching the capillaries venules and veins during exercise because of the marked precapillary dilatation for the tissue volume remained essentially the same in the perfusion experiments where the venous pressure was unchanged and the lowered arterial pressure prevented the capillary pressure from rising

Sharpey Schafer (1963) has put forward results which he interpreted as evidence that venous constriction occurs immediately after exercise both in normal and in sympathectomized human forearms These findings seem to contradict those made in the present study This may be explained by the fact that the method of Scharpey Schafer does not measure constriction and dilatation of the veins as such but venous tone in mm Hg per ml — which is thus an expression of the volume elasticity of the veins The elasticity values change with volume since the pressure/volume curve for the veins is not a straight line and elasticity is increased (venous tone is increased) at higher venous volumes (see e g Gauer and Thron 1962) To check whether this might explain the discrepancy the volume elasticity of the veins was calculated using the distensions of the veins induced by all CFC determinations in ten consecutive experiments The mean elasticity value during rest was 12 (mm Hg × 100 g)/ml (44 determinations) and increased to 18 during exercise with one contraction per second (20 determinations) and to 19 during two contractions per second (13 determinations) Thus in these experiments there was a fairly small but consistent increase of elasticity (decrease of distensibility) when the veins in fact increased their volume In contrast there was no change of venous elasticity during exercise in the nine experiments with constant blood flow — where the regional blood volume did not change — venous elasticity during rest was 14 (32 determinations) and during exercise 14 (mm Hg × × 100 g)/ml (38 determinations)

Thus it appears that the venous distensibility does not change during exercise provided the determinations are performed starting from the same venous volume as during rest This is in accordance with the results of Kidd and Lyons (1958) who avoided variations in initial venous volume by applying a high venous pressure before the beginning of exercise

The second slow phase of the volume change during exercise might theoretically be attributed to a gradual further relaxation of the capacitance vessels or to a movement of fluid into the tissue The first possibility seems unlikely since it would imply completely different time constants for the relaxation of the capacitance vessels and the resistance vessels The possibility is ruled out at least as the only explanation by the fact that the tissue volume can increase by as much as 10 per cent during this type of exercise (Jacobsson and Kjellmer 1964) while in the present investigation it was not possible to increase tissue volume by more than a small fraction of this volume even with very high distending pressures A pressure of 30 mm Hg applied to the venous side — far in excess of the slight increments in mean venous pressure occurring as a

result of exercise vasodilatation — increased the regional tissue volume by at most 2—3 per cent in these experiments.

Thus it appears that the increase of volume is due to a loss of fluid from the vessels to the tissues. This transudation of fluid was studied already by Ranke (1865) who found that the water content of the tetanized frog muscle increased while that of the blood decreased. He concluded that the extra water in the tissues is derived from the circulating blood and he showed that it cannot be accounted for by the water liberated during the oxidation processes. These findings were later confirmed in mammals (Barcroft and Kato 1915, Keys and Taylor 1935).

The transport of fluid into the tissue has been shown to be dependent on the capillary hydrostatic pressure and can be abolished if the pressure is low enough (Fig. 4 and 7). This is in line with a filtration process. If instead the transfer were due to diffusion, the volume changes could not have been altered unless the osmotic forces governing diffusion had been changed.

The driving force for the net capillary outward filtration during exercise must be an increased mean capillary hydrostatic pressure. Other factors which might, theoretically cause an outward filtration are a decrease of plasma colloid osmotic pressure, an increase of tissue fluid colloid osmotic pressure or a decrease of tissue pressure. None of these changes appears to be plausible under the present experimental conditions. An increase of the capillary hydrostatic pressure, on the other hand, is known to occur when vasodilatation is brought about (Landis 1934).

The elevation of mean capillary pressure calculated from these experiments is related to the degree of vasodilatation, which in turn is dependent on the severity of exercise (Table I). When the arterial and venous pressures are kept constant, a rise in capillary pressure is brought about by a decrease of the r r ratio. This means that the precapillary resistance vessels are relatively more dilated than the postcapillary. It has been pointed out above that the venous pressure rose somewhat during the exercise hyperemia as an artifact, but this only contributed with 1 or at most 2 mm Hg to the rise of capillary pressure. Thus it does not invalidate the conclusion that the precapillary resistance vessels are more markedly affected by the exercise metabolites than are the postcapillary.

The capillary filtration coefficients (CFCs) found here agree with values found previously in resting skeletal muscles (Pappenheimer, Renkin and Borrero 1951, Mellander 1960, Cobbold *et al.* 1963[1]). Kjellmer 1964 reported a three- to fourfold increase of the CFC values for the human forearm during severe exercise. The changes reported here are of the same magnitude.

An increase of the CFC may be due to an increase in the capillary surface area available to blood flow and/or an increase of capillary permeability. These two factors are impossible to separate directly but it might be useful to consider the results of experiments where diffusion was studied instead of filtration. Renkin (1959) measured the diffusion rate of radioactive potassium from the capillaries into the gracilis muscles of dogs and determined what he called the "permeability-surface area product". This was found to increase to three-fold its original level during rhythmic contractions of the muscle.

The rate of diffusion is determined by Fick's law of diffusion: rate of diffusion = constant × concentration gradient × area/length, while filtration is considered to be

[1] Some of the present results were included in the study by Cobbold et al.

determined by Poiseuille s law (rate of filtration = constant × pressure gradient × × radius^4/length) With the pore theory of capillary function (cf Renkin and Pappenheimer 1957) in mind an increase of the rate of diffusion for a non lipoid soluble sub stance such as potassium might with the same driving force occur in two ways 1 the capillary surface area could be unchanged and each pore could increase its diameter (i. e an increase of permeability) 2 the capillary surface area could increase without any change in pore diameter For a threefold increase in rate of diffusion the first possibility requires a ninefold increase of the CFC while the second possibility requires only a threefold increase of CFC In the present experiments the CFC was never observed to increase to more than three to fourfold its original level which makes it probable that the change was due to an increase in capillary surface area rather than to changes in pore size Direct determinations of the permeability characteristics of the capillary membrane in resting and contracting calf muscles of dogs strongly support the view that permeability does not change during exercise (Arturson and Kjellmer 1964) It thus seems justified to conclude that the capillary surface area open to blood flow in creases up to three to fourfold during severe exercise

From the work of Krogh (1929) it is known that only a fraction of the capillaries in the resting skeletal muscles at a given moment is open to the blood flow presumably due to intermittent closure of the capillaries by their sphincters Krogh also showed that the fraction of capillaries open to blood flow increased markedly during muscle contractions in fact often as much as ten to fiftyfold The data reported here are of course quantitatively not directly comparable with Krogh s since one set of data refers to capillary surface area and the other one to capillary counts But assuming the capillaries to be of about the same length the discrepancy seems large enough It would seem that Krogh s data should not be considered *quantitatively* reliable for two reasons first owing to uneven distribution of blood flow the india ink injection technique is bound to underestimate the number of patent capillaries (as indeed is illustrated by the fact that in some resting muscles Krogh did not find any capillaries open at all) and second his data on volume of blood contained in the capillary bed suggest that the total capillary volume was grossly overestimated (10 per cent of the muscle volume is taken up by the capillaries in the dog gastrocnemius muscle during maximum dilata tion according to Krogh) The data of Martin Woolley and Miller (1932) obtained on cats show that the capillary counts in the gastrocnemius muscle increase roughly threefold when contracting muscles are compared with resting ones values which agree well with the increase of CFC found in the present study

If it is assumed that each precapillary sphincter controls blood flow through a capil lary area of approximately the same magnitude a threefold increase of CFC should correspond to about a threefold increase in the number of open sphincters Opening up of new capillaries is accompanied by an increase of the blood flow up to about six fold its original level Even if the higher blood flow spreads over a larger capillary area the linear velocity of the blood must be approximately doubled and the time available *for exchange with the tissue approximately halved* provided the capillaries are not distended or elongated markedly during the vasodilatation This might be of signifi cance in the exchange of substances across the capillary wall during exercise

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From the Department of Physiology University of Goteborg Sweden

An Indirect Method for Estimating Tissue Pressure with Special Reference to Tissue Pressure in Muscle during Exercise[1]

By

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Abstract

Kjellmer I *An indirect method for estimating tissue pressure with special reference to tissue pressure in muscle during exercise* Acta physiol scand 1964 62 31—40 — Tissue pressure is one of the determinants of capillary filtration and appears to be a major limiting factor of filtration exchange during muscular exercise Since direct measurement of tissue pressure through a needle was considered impossible an indirect method was developed to record tissue pressure in skeletal muscle during rest and exercise This method is based on the fact that veins are normally collapsible and that the pressure inside patent veins can never be less than the extramural pressure i e tissue pressure When the tissue pressure exceeds the central venous pressure the pressure in the local veins is dictated by and becomes a measure of the tissue pressure Instead of measuring this pressure the changes in volume of the muscle were recorded plethysmographically and the pressure below which changes of central venous pressure did not influence volume was noted This pressure was regarded as corresponding to the minimum tissue pressure The maximum tissue pressure was obtained from distensibility curves for the veins The accuracy of the method is about [illegible] cm H_2O During rest the tissue pressure was only little above zero and rose during exercise in relation to the amount of filtrate accumulated in the muscle

Though the hydrostatic pressure of the tissue fluid (here called the tissue pressure) is normally considered to be very low it cannot always be ignored in the study of the regulation of net filtration exchange Thus it has been claimed on indirect grounds to rise as much as 35 cm H_2O in human forearms during venous congestion and thereby limit the effect of a raised capillary pressure (Landis and Gibbon 1933)

[1] Part of this study was reported before the XI Scandinavian Physiological Congress in Copenhagen August 1963

Studies on the isolated calf of the cat suggested that tissue pressure increased as a *result of an accumulation of capillary filtrate during prolonged muscular exercise* and that this increase was the factor mainly responsible for limiting filtration (Jacobsson and Kjellmer 1964) Others working with the same type of preparation however concluded that tissue pressure does not change until the tissues are grossly edematous (Pappenheimer and Soto Rivera 1948) This study was undertaken to ascertain in a more direct way, whether tissue pressure increases when filtrate accumulates in exercising muscle and if so the relationship between the tissue pressure and the tissue volume

Judging from the literature tissue pressure is usually measured via a thin needle inserted in the tissue and measuring either the equilibrium pressure of a small quantity of fluid injected into the tissue (e g Swann Montgomery and Lowry 1951) or the minimum pressure necessary to force a small amount of fluid into the tissue (e g Wells Youmans and Miller 1938) This group of methods however has been criticized — even by those who used them (McMaster 1946 and others) The objections raised against the method are first even the smallest needles are several hundred times larger than the spaces where they should record the pressure (Guyton 1963) and second the difficulties encountered when one tries by means of fluid movements to measure pressure in a substance of semi solid gel character like the interstitial fluid (cf Rusznyák et al 1960 p 340)

To avoid the difficulties involved by the needle methods direct readings of pressures in small veins have been used as a measure of tissue pressure under certain experimental conditions (Ryder Molle and Ferris 1944) This method has the advantage of determining pressure in a true fluid but its use is limited by the fact that it requires elimination of any influence of the central venous pressure and that it may be technically difficult to measure the pressure in small veins without wedging the catheter However the method to be described may in fact be considered an elaboration of this method

While the present experiments were in progress a completely new approach was described by Guyton (1963) who measured the pressure inside small perforated capsules implanted in the tissues Guyton arrived at the surprising conclusion that the tissue pressure is normally subatmospheric (negative) The capsule technique was not considered to be entirely suitable for the present purpose partly because Guyton reported technical difficulties when implanting the capsules inside skeletal muscles partly for theoretical considerations (see below Discussion)

Thus none of the methods hitherto described appear particularly suitable for recording tissue pressure in skeletal muscle during rest and exercise It was therefore decided to try an indirect approach based on the following considerations 1 Flow through a collapsible tube can occur only when the pressure inside the tube is at least *as high as that outside* 2 *Venules and veins are collapsible tubes* 3 Tissue pressure acts directly on the venules and veins The intravascular pressure in venules and veins can never be lower than tissue pressure if blood flow is to be preserved as has been shown experimentally for different tissues such as the skin of the forearm the kidney the lung and the eye (Ryder et al 1944 Swann et al 1951 Permutt Bromberger Barnea and Bane 1962 and Bill 1962)

For convenience the pressure in the veins inside the organ studied will be called local venous pressure (LVP) that in the veins outside the organ central venous pressure (CVP) i e abbrevations used though in a slightly different sence by Ryder et al (1944) Since tissue pressure can never exceed the LVP without stopping blood flow

the LVP is determined by the magnitude of the tissue pressure as soon as this exceeds the CVP and secondly there must be a steep pressure drop in a vein when it passes the boundaries of an organ with a tissue pressure exceeding the CVP. This steep pressure drop has aptly been termed the "vascular waterfall" (Permutt et al. 1962). The waterfall metaphor is appropriate in several respects: it stresses that the pressure drop in a vein emerging from an organ with a high tissue pressure is steep and that neither LVP nor blood flow can be changed by modifying the CVP until the CVP exceeds tissue pressure, i.e. until the waterfall is abolished.

The present method estimates the tissue pressure from the level at which the waterfall effect is overcome when the CVP is raised stepwise from a low value.

Methods

Model experiments

To test the theoretical validity of the method some model experiments were performed. The model was built to study the pressure within a collapsible tube through which water was conducted. The tube consisted of a length of thin latex tubing connected at both ends to rigid tubes. Water was conducted through the collapsible tube from the arterial inflow to the venous outlet. Both inflow and outflow pressures were adjustable. The tube was placed in a water filled plethysmograph. The pressure within the system was measured with a catheter connected to a low pressure variable inductance transducer (Elema, Sweden). The tip of the catheter could be placed either inside the tube or in the rigid outlet tube. Any change of volume in the plethysmograph was recorded with a small piston recorder. To the lever arm of this recorder was attached a thin metal blade which moved between an electric bulb and a photo-resistor so that the shaded area of the photo-resistor changed every time the piston recorder moved. The photo-resistor was connected to a bridge circuit and the output fed into a Sanborn Twin Viso recorder, the other channel of which recorded the pressure.

In this model the pressure inside the flaccid latex tube corresponded to the LVP, the pressure in the rigid outlet tube to the CVP and the height of the water column in the plethysmograph above the collapsible tube to the tissue pressure.

Animal experiments

Seventeen cats weighing 2.3 to 5.5 kg were used. After ether induction chloralose 70 mg/kg b.w. was given i.v. The preparation, which has been described in detail elsewhere (Kjellmer 1964), is briefly as follows. The calf muscles were isolated from the rest of the body so that the sole connections were the femur and the popliteal artery and vein. The paw was removed, the skin veins were ligated, the bone marrow cavity of the femur was plugged and all branches of the popliteal vessels were ligated to ensure that the total blood flow through the lower leg was conducted through the main vessels and that blood flow from tissues other than skeletal muscles was reduced to a minimum.

The calf was placed in a plethysmograph with the skin of the thigh used as a watertight seal. The height of the water column was 5 to 9 cm above the centre of the tissue mass. In four experiments the normal funnel of the plethysmograph was replaced by a high one so that it was possible to obtain a water column up to 30 cm above the tissue.

Four parameters were recorded. 1. Arterial pressure in the contralateral femoral artery was measured with a mercury manometer. 2. Venous outflow pressure in the popliteal vein was measured with a water manometer. The zero level of the manometer was adjusted to the centre of the tissue mass. The pressure recorded corresponds to the CVP. 3. Venous outflow from the popliteal vein was measured with a drop chamber and a [illegible] unit. The outflow pressure could be varied by adjusting the outlet level from the drop chamber. 4. Changes in tissue volume were measured with a piston recorder connected to the plethysmograph.

The peripheral end of the divided sciatic nerve was stimulated with pulses 3 V, 0.1 msec at varying frequencies from a Grass model S4 GR stimulator to produce muscular work and exercise hyperemia. All recordings were made on a slowly moving smoked kymograph paper. The venous pressures were not registered continuously but read at suitable intervals.

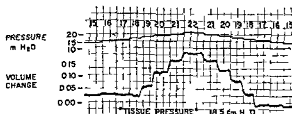


Fig 1 Model experiment showing volume changes (arbitrary units) occurring when outflow pressure (CVP) is changed stepwise Hydrostatic extramural pressure (tissue pressure) set at 18 5 cm H O

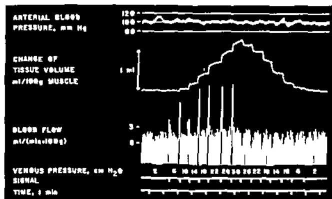


Fig 2 Cat 4 7kg Changes in tissue volume and blood flow occurring when venous outflow pressure (CVP) is changed stepwise Water pressure in plethysmograph 8 cm

Results

Model experiments

When the tissue pressure in the plethysmograph was set at a higher level than CVP the pressure measured inside the collapsible tube was the same as the tissue pressure and when the catheter was withdrawn into the outflow tube the pressure suddenly dropped to the level of the CVP when the catheter tip passed the connection between the flaccid and the rigid tube

The recordings of pressure in the collapsible tube (LVP) during stepwise changes of the CVP showed that LVP did not change with CVP until the latter pressure exceeded tissue pressure

To mimic the animal experiments the change in volume of the collapsible tube was recorded together with CVP which was altered stepwise Fig 1 shows an example where tissue pressure was set at 18 5 cm H O and the CVP raised 1 cm at a time from 15 to 22 cm H O The volume remained unchanged until the CVP was raised from 18 to 19 cm H O and when the CVP was lowered no change occurred below the step between 18 and 17 cm H_2O This record is typical in two respects — it shows a fairly close correlation between the tissue pressure and the level at which changes in the CVP begin to influence the volume and it shows that there can be some difference between the critical values obtained when raising and lowering the CVP This difference was never larger than 1 cm H_2O

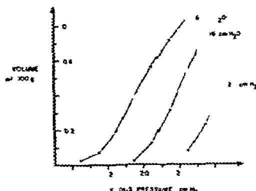


Fig 3 Cat 4 0 kg Pressure volume relationship obtained by raising venous outflow pressure (CVP) at three pressure levels in the plethysmograph

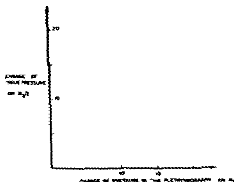


Fig 4 Relation between estimated change of tissue pressure and change of water pressure in plethysmograph in four experiments where the height of the water column was varied Solid symbols = change of maximum tissue pressure hollow symbols = change of minimum tissue pressure Dotted line = line of identity For definitions of maximum and minimum tissue pressure see below

Animal experiments

In the animal experiments the volume was changed by stepwise alteration of the CVP In order to produce volume changes large enough to be measured accurately the CVP was raised 2—4 cm H O at a time

Fig 2 gives an example where CVP was changed 4 cm H O at a time from 2 cm H O In this case the height of the water column in the plethysmograph was 8 cm above the centre of the tissue mass Note the similarity between the volume changes in this case and the model experiment in Fig 1 In the animal experiment there was also one level below which a change of CVP did not influence the volume Above this level the volume changed stepwise with the CVP

In each case it was possible to find a CVP level below which changes of pressure produced no change in volume The threshold pressure was taken as the midpoint in the first step that produced a change in volume The threshold pressure was usually close to and occasionally somewhat lower than the water pressure in the plethysmograph In Fig 2 the mean height of the water column was 8 cm as was that of the threshold pressure

In order to study the relationship between the pressure volume curve and the water pressure in the plethysmograph the latter was varied between 6 and 26 cm H O using

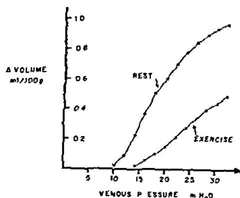


Fig 5 Cat 4 1 kg Pressure volume relationship obtained by raising the venous outflow pressure (CVP) during rest and during exercise when the muscles have increased 10 per cent in weight Water pressure in plethysmograph 9 cm

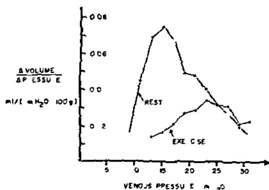


Fig 6 Cat 4 1 kg The values in Fig 5 are replotted to show the relationship between venous distensibility and pressure

the high funnel on the plethysmograph Pressure volume curves were recorded repeatedly at various water pressures The curves were constructed from data obtained from records like the one shown in Fig 2 The rapid change in volume produced by each increment of the CVP was measured before the subsequent slow change in volume due to capillary filtration The total volume changes were then plotted against the pressure Fig 3 shows such pressure volume curves obtained in one experiment at water pressures of 6 16 and 26 cm The curves shifted to the right in rough proportion to the increment in water pressure In four experiments the water pressure was varied in the plethysmograph In each case the pressure volume curves shifted in proportion to the change in water pressure Fig 4 shows the relationship between the extra pressure applied in the plethysmograph and the shift of the pressure volume curve along the pressure axis The correlation is so close that it seems justified to conclude that the water pressure in the plethysmograph is transmitted to the tissue without decrement

Fig 5 shows the pressure volume relation in an experiment where the water pressure was 9 cm Curves were obtained during rest and when the muscle volume had increased 10 per cent during prolonged exercise It is obvious that the exercise curve has shifted to the right of the control curve but this displacement is only slight If however the curves are rearranged so that the distensibility is instead plotted against venous pres-

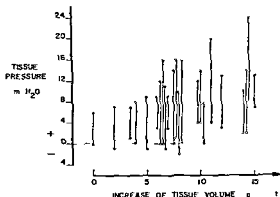


Fig 7 Relationsh p between tissue pressure and tissue volume In each case the maximum and mini num tissue p essures a e given Values at normal tissue volume are mean alues from 15 cats Hollow symbols denote single determ nat ons on 10 cats during exercise solid symbols s ngle determinations on 2 cats after intra arterial infusion of h sta nine

sure another difference betv een the two curves b comes apparent (Fig 6) Both curves have an ascending and a descending limb The ascending limb of the exercise curve is less steep and covers a wider pressure range than the curve obtained during rest If the threshold pressure is taken to indicate the minimum tissue pressure and the pressure corresponding to the peak value of distensibility taken to indicate the maximum tissue pressure (see Discussion) then in Fig 6 the maximum and minimum pressures during rest are 15 and 9 cm H_2O respectively while the corresponding pressures obtained during exercise are 23 and 13 cm H O During exercise the tissue pressure thus increased particularly the maximum pressure so that the unevenness of distribution had become accentuated This was a typical finding

The tissue pressures determined in this way are composed of the true tissue pressure and the extra pressure exerted by the water column in the plethysmograph To calculate the true tissue pressure the mean water pressure was subtracted from the points of maximum and minimum pressures of the distensibility curves The values of maximum and minimum pressures calculated from the curve obtained during rest in Fig 6 are accordingly 15 — 9 = 6 cm H O and 9 — 9 = 0 cm H O

In 15 experiments the mean maximum and minimum pressures were 5 9 (2 to 13 cm H_2O) and — 0 2 (— 2 to + 5 cm H O) respectively In two cases no change of tissue pressure could be observed when the volume increased during exercise (increase of volume 7 and 10 per cent respectively) In all the other experiments there was a clear increase of tissue pressure with increasing volume Fig 7 shows the distribution of tissue pressure in resting muscles and after accumulation of increasing amounts of capillary filtrate in ten cats during exercise and two cats during intra arterial infusions of histamine (the two cats where tissue pressure did not change when volume increased are not included in the figure) From this figure it seems as if the highest tissue pressure increases gradually with volume while the minimum pressure does not begin to increase until the muscles are markedly swollen

Discussion

The method used in based on the assumptions that the veins are normally collapsible so that flow ceases wh n extravascular pressure exceeds intravascular pressure and that the extramural pressure that tends to collapse the veins is the same as the tissue pres-

sure These assumptions have been strengthened experimentally by Ryder et al (1944) both for excised veins and in vivo for human arm veins They showed that the veins were freely collapsible and that the hydrostatic pressure exerted by the tissues was completely transmitted to the walls of the veins Fig 3 and 4 show that increments of the height of the water column in the plethysmograph were transmitted quantitatively to the tissues and recorded as changes of tissue pressure by the present method One may therefore conclude that the assumptions are valid also in this case and that the present method measures changes of tissue pressure with an accuracy of about 2 cm H_2O

It is apparent from Fig 6 that exercise not only increased the threshold pressure but also changed the pressure volume relationship so that the ascending limb of the distensibility curve covered a much wider pressure range during exercise than during rest. In view of the results obtained both on isolated veins (Clark 1933) and from in vivo experiments (e g Gauer and Thron 1962) it is difficult to believe that the ascending limb represents a true increase of venous distensibility with increasing pressures Instead it might represent misleading determinations due to uneven distribution of tissue pressure The curves shown in Fig 6 may thus be explained if one assumes that the lowest tissue pressure is represented by the threshold pressure i e at which the volume begins to increase and when the central venous pressure is increased further veins in areas with higher tissue pressure are gradually distended until the highest tissue pressure has been overcome at the point where the distensibility curve reaches its peak According to this interpretation each distensibility curve would thus allow estimation of both the maximum and the minimum pressures in the tissues

To obtain the true tissue pressure the mean pressure exerted by the water was subtracted from the points of maximum and minimum pressure Usually the difference thus obtained between maximum and minimum tissue pressure was about 6 cm H_2O during rest (cf mean values from 15 experiments in Fig 7) This distribution of tissue pressure might be due to differences between various tissues such as muscles connective tissue and fat or to differences between various muscle groups (see below) The tissue pressures in resting tissues are thus only slightly above zero pressure This is in agreement with most previous findings obtained with needle methods (e g McMaster 1946 Wells et al 1938) or the indirect method of Ryder et al (1944) but there is a large discrepancy between these findings and those of Guyton who claims the tissue pressure to be well below zero in resting muscles Besides the difficulties associated with the artificial situation created when a large capsule is permanently implanted in the tissue particularly regarding the dynamics of protein and fluid transfer across the newformed tissue membrane some consequences of the negative tissue pressure do not seem to apply to the normal behaviour of skeletal muscles The same experiments (Guyton 1963) showed the tissue space to have a very high modulus of volume elasticity in the negative tissue pressure range and a very low modulus when tissue pressure becomes positive This should cause the pressure curve to rise very rapidly when the tissue pressure is in the negative range but extremely slowly when in the positive range Such an arrangement would be teleologically unsatisfactory at least for skeletal muscles because it would effectively prevent any gross net exchange of fluid between the circulating blood and the largest tissue mass in the body and thus prevent the extra cellular fluid from acting as a buffer minimizing changes of blood volume There is considerable experimental evidence that instead suggests that changes of tissue pressure do not come into play until tissue volume has changed markedly a reduction of

capillary pressure during bleeding causes a progressive decrease of muscle volume (Öberg 1963) and a hemodilution due to reabsorption of interstitial fluid in an amount corresponding to about two thirds of the plasma lost (Kaufmann et al 1956) On the other hand an increase of capillary pressure caused by venous congestion or exercise leads to continuous outward filtration the rate of which is at first steady and then declines This is in accord with the view that the more the tissue volume is increased the more rapidly the tissue pressure rises i e that the modulus of elasticity increases at higher volumes

The relationship between tissue pressure and tissue volume found with the present method seems to be more in accord with what might be expected from the above mentioned findings Here one finds a low modulus of elasticity that seems to increase gradually with swelling of the muscles This is the type of curve one would expect from a system like the skeletal muscle which is enclosed in a fascia that contains both elastic fibres and relatively unelastic collagen fibres

The thickness of the fascia differs from muscle to muscle Wells et al (1938) recorded intramuscular pressure in the gastrocnemius and the soleus with a needle and found that the pressure in the soleus could rise to 50 cm H_2O during venous congestion while the pressure in the gastrocnemius never rose above 20 cm H_2O These findings were ascribed to differences in the surrounding fascia The marked tendency towards an uneven distribution of tissue pressure in the muscle mass investigated here might likewise be attributed to differences in tissue pressure between various muscles in the course of their swelling

To ascertain whether the change of tissue pressure during exercise was caused mainly by the muscular contractions *per se* or by the accumulation of capillary filtrate in the muscles tissue pressure was determined during rest and when the volume had increased during intra arterial infusions of histamine The values are included in Fig 7 The relationship between pressure and volume change seems to be the same as during exercise Moreover in three cases it was possible to obtain a reading of the tissue pressure immediately after exercise and the values then obtained were the same as during exercise In most cases however it was impossible to make such determinations since muscle volume usually fell so rapidly after exercise that muscle volume (and thus tissue pressure) decreased materially during the period necessary for estimation of tissue pressure The three values obtained immediately after exercise together with the values obtained during administration of histamine strongly suggest that the important determinant of tissue pressure is the degree of volume increment during exercise rather than the contractions themselves Beyond doubt a muscle contraction produces a rapid and marked increase of tissue pressure but with the type of contractions used this rise of pressure is apparently so transient that it does not significantly influence the present type of recording

This study was prompted by the finding that the mean capillary pressure rose by 5—15 mm Hg during exercise (Kjellmer 1964) and that opposing forces tended to counteract the effect of a raised capillary pressure and set a limit to outward filtration In an attempt to analyze the nature of the opposing forces it was pointed out that the outward filtration tended to concentrate the intravascular proteins and dilute the proteins in the interstitial space and thereby increase the gradient of colloid osmotic pressure across the capillary wall by at most 3—4 mm Hg (Jacobsson and Kjellmer 1964) It was also concluded that the tissue pressure was the most important limiting factor The present results seem to corroborate this view Even if it is not possible to express the

change of tissue pressure during exercise by a single value e g a mean value it is obvious from Fig 7 that tissue pressure rises when filtrate collects in the muscle and second that the increase in tissue pressure is large enough substantially to counterbalance the raised capillary pressure

This study was supported by grants from the Faculty of Medicine University of Goteborg from Svenska idrottens vetenskapliga forskningsråd from School of Aerospace Medicine AFSC through the European Office Aerospace Research United States Air Force (Grant AF EOAR 61-47) and from U S Public Health Service (Grant HE 05675 03)

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Acta physiol scand 1964 62 41—45

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Capillary Permeability in Skeletal Muscle during Rest and Activity

By

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Abstract

Arturson G and I Kjellmer *Capillary permeab lity in skeletal muscle during rest and activity* Acta physiol scand 1964 62 41—45 — In exerci ng skeletal muscle fluid is filtered from the blood stream to the interstitial space The filtrat on process is facilitated by an increase of the cap llary filtration coefficient This coeff ent is *dependent* upon cap llary surface area and cap llary permeab l ty The present study was designed to ascerta n whether the cap llary permeability increases in exerc sing muscle The permeabil ty of the capillary membrane a studied in two dogs D xtran of d fferent molecular sizes as injected intravenou ly after which the concentration of th dextran in pure muscl lymph as compared with that in arterial plasma The rat o bet een these *concentrations* d d not change s gn ficantl when exercise was s mulated by rep ated st mulation of the motor nerve It is therefo e concluded that cap ll ry permeability does not change during muscular exercise

During muscular exercise fluid in the circulating plasma is lost to the interstices of the active muscles This well known edema formation has been sho n to depend mainly on filtration due to a raised capillary hydrostatic pressure and to be augmented by an increase of the capillary filtration coefficient (Kjellmer 1964) The capillary filtration coefficient is an expression of the amount of fluid filtered per minute per 100 g tissue per mm Hg change in capillary pressure Any increase in the filtration coefficient is an expression of an increase of capillary permeability and/or an increase of the perfused capillary surface area

To differentiate bet een changes in permeability and surface area one of these t o variables namely capillary permeability was stud ed in the present xperiments in which the sieving characteristics of the capillary membrane ere determined

The permeability of the capillary wall has been claimed to increase in response to a variety of conditions incl ding hypoxia at least if se ere Land s 1928 Hendley and

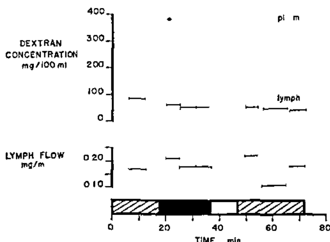


Fig 1 Dog 15 kg Lymph flow and dextran concentration in plasma and lymph during rest + passive movements (shaded) and during muscular contractions + passive movements (black) Blank field = interval

Schiller 1954) and distension of the capillary wall (stretched pore phenomenon Wasserman Loeb and Mayerson 1955) These phenomena as well as bradykinin which has been suggested as the active vasodilator during muscular exercise (see *e g* Dornhorst 1963) and which is known to increase capillary permeability (Elliott Horton and Lewis 1960) might affect the permeability of the capillary wall during exercise It was therefore considered legitimate to determine the capillary permeability in exercising muscle

If the permeability substantially increased during exercise proteins would leak through the capillary wall Two observations argue strongly against leakage of extra proteins during exercise first neither the total concentration of proteins nor the fraction of albumin in mu cle lymph changes during exercise (Jacobsson and Kjellmer 1964 a) and second the filtrate accumulated in the muscles during exercise seems to be poor in proteins since after exercise it is reabsorbed even when the lymphatics are blocked (Jacobsson and Kjellmer 1964 b) However changes in capillary permeability too small to affect the protein transport but large enough to influence the filtration coefficient might still occur

Methods

Two dogs less than one year old and weighing 15 and 16 kg were used Nembutal 30 mg per kg b w was administered and small amounts were added during the exper ment Atropine 1 mg was given i v and the dogs were intubated The abdomen was opened by a midline incision and both renal pedicles were clamped 10 ml p r kg b w of a 6 per cent dextran solution in 0 9 per cent saline was then injected i v The dextran used (Pharmacia AB) had an average molecular weight $\overline{M}_w$ of 40 400 (range 10 000—80 000)

After injection of the dextran the dog was left for about 4 hours The blood pressure recorded with a mercury manometer in a brachial artery was stabl around 130 mm Hg in both experiments

A small amount of Patent Blue Violet in 11 per cent solution was injected into the skin of the paw By blunt dissection the deep lymph vessels accompanying the femoral blood vessels were then freed 4—5 cm above the knee All vessels were l gated and one was cannulated with a PE-50 polyethylene catheter (Clay Adams Inc) The circulation of the paw was then care

1 This dextran as generously supplied by Pharmacia AB Sweden

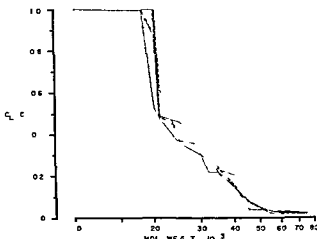


Fig 2 Dog 15 kg Lymph/plasma concentration ratio (C_L/C_P) for different molecular sizes of dextran in two samples obtained during rest + passive movements (continuous lines) and two samples obtained during muscular contractions + passive movements (broken lines) Abscissa in log scale

fully shut off with tight ligatures around the ankle To secure a measurable lymph flow during rest the ankle joint was flexed and extended in a standardized way once a second

The sciatic nerve was cut and was stimulated from a Grass stimulator Model S4CR through bipolar silver electrodes with supramaximal stimuli at a frequency of 2/sec

The muscle lymph was sampled in previously weighed small test tubes Immediately before the middle of the lymph sampling period a blood sample was drawn from the contralateral femoral artery

Some samples were first collected during rest In order to secure fluid representative of the limb during exercise sampling was not commenced until some minutes after the exercise had been started

The lymph and plasma samples were analyzed for total dextran concentration with the anthrone method (Wallenius 1954) and the distribution of molecular weight was determined using the method described by Wallenius *et al* (1960) This method determines turbidimetrically the precipitation curve obtained when ethanol is slowly added to the water solution of dextran Since the largest dextran molecules are precipitated first the resulting curve can be converted into a molecular weight distribution curve For discussion and evaluation of the method see Wallenius *et al* (1960) and Arturson (1961)

Results

The results are summarized in Fig 1 and 2 which show the results noted in one dog but are representative of both experiments Fig 1 shows data obtained on lymph flow and on concentration of dextran in plasma and lymph which is shown to diminish almost linearly with time When exercise was induced lymph flow increased and reached a peak during the first few minutes after which it returned towards resting values In order to be sure that the catheter had been flushed with lymph produced during exercise lymph sampling was not started until after the peak flow had passed The peak lymph flow immediately after the beginning of exercise is not shown in the figure

For each molecular size of dextran the ratio between the concentration in lymph and that in the corresponding plasma sample was calculated and called the C_L/C_P ratio Fig 2 shows the curves for the C_L/C_P ratio plotted against the molecular weight of the fraction As can be seen from the figure exercise had no effect on the curves

Discussion

The capillary wall acts as a barrier between blood and tissue through which solutes and solubles are transported by diffusion and filtration Substances passing through the capillary membrane in a water phase are thought to have only a small fraction of the capillary surface area available for passage and their passage is restricted if their molecular size is too large (*cf* Renkin and Pappenheimer 1957)

The ability of the membrane to retain large molecules can be measured by determining the sieving characteristics of the membrane This in turn is a measure of the capillary permeability Grotte (1956) showed with the aid of the same technique as in the present study that the capillary permeability differs from region to region e g much higher in the liver than in the tissues drained by the leg lymphatics Such a difference in capillary permeability between various organs was also demonstrated by Mayerson *et al* (1960)

Owing to the regional differences in capillary permeability in a study of the present kind care should be taken that the lymph is drained solely from one particular type of tissue — in this case striated muscle Since the paw is drained to a fair extent by the deep lymphatics of the leg while the skin above the ankle seems to be drained almost exclusively by the superficial lymphatics cannulation of a deep lymph stem near the knee region after exclusion of the paw from the circulation enables collection of almost pure lymph from the calf muscles (Jacobsson and Kjellmer 1964 a)

To measure the sieving characteristics of the capillary membrane in skeletal muscle dextran injected intravenously was allowed to spread in the whole organism for about 4 hours before lymph sampling was started This time should be sufficient to ensure steady state conditions (*cf* Grotte 1956)

The sieving characteristics of the capillary wall determine the size of the dextran molecules that pass freely into the lymph and the size of the molecules totally retained in the plasma Dextran fractions of intermediate molecular weight have been shown to pass the membrane by restricted diffusion (Grotte 1956)

In the present experiments it was found that the ratio between the concentration of various fractions of dextran in muscle lymph and the concentration in plasma was not affected by exercise (Fig 2) — i e the sieving characteristics of the membrane were not changed

It might be expected that the C_L/C_P curves in Fig 2 should shift to the left if capillary permeability were unchanged and filtration rate increased It has been theoretically deduced (Pappenheimer Renkin and Borrero 1951) and experimentally shown (Grotte 1956) that molecules passing the capillary wall by the process of restricted diffusion should appear in lower concentrations in the filtrate when the rate of filtration is increased Furthermore the sieving effect is more pronounced for larger than for smaller molecules This would lead to a decrease of the overall concentration of dextran in filtrate and in lymph and simultaneously a shift of the curve for the molecular weight distribution towards dextran fractions of lower molecular weight

In these experiments no such changes of the dextran composition of muscle lymph were observed during exercise in spite of the fact that the filtration rate must have increased The reason for this is probably a quantitative one To obtain a clearcut change of the concentration and distribution curve for dextran in lymph Grotte had to elevate the venous pressure to 50 mm Hg The lymph flow then increased to twice the resting value In the present experiments neither the filtration rate nor the change of capillary

pressure was measured For a rough comparison however it might be permissible to compare Grotte s data with those obtained on cats (Kjellmer 1964) where an exercise of 2 single twitches per sec (the same frequency as in the present series) which caused almost maximal vasodilatation in the muscles was shown to increase the hydrostatic mean capillary pressure about 14 mm Hg This should be compared with the figure of 50 mm Hg venous pressure (corresponding to a change of mean capillary pressure of some 40 to 45 mm Hg) in Grotte s experiments Lymph flow in the present experiments did not increase significantly during exercise (except in the initial phase of exercise which was not included in the sampling period) This is also in accord with the view that the change of filtration rate in these experiments was below that needed to produce a detectable effect on the dextran composition of the muscle lymph

It may thus be concluded that the capillary permeability in skeletal muscle was not measurably changed during simulated exercise

This study was supported by grants from the Defence Medical Board [illegible] Research Council (G A) and from the Faculty of Medicine University of [illegible] The investigation has also been sponsored in part by School of Aerospace M[illegible] through the European Office Aerospace Research United States Air Force (Grant AF EOAR 61—47) and by U S Public Health Service (Grant HE-[illegible]-03)

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Acta physiol scand 1964 62 46—50

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Cyclic Variation in the Body Temperature of the Male Rabbit

By

G DEGERMAN and J E KIHLSTROM

Received 20 January 1964

Abstract

G Degerman and J E Kihlstrom *Cyclic variation in the body temperature of the male rabbit* Acta physiol scand 1964 62 46—50 — Daily recordings of body temperature in male rabbits have been shown to vary cyclically The mean length of these cycles is very close to that of the cyclic variations in the sexual functions of the male rabbit previously found Further the *coincidence in phase between the cycles of the body temperature and those of the seminal volumes* is statistically significant

The biphasic thermal variation during the menstrual cycle in women first observed by van de Velde (1904) is well known Similar variations in body temperature during the estrus cycle have been reported in females of cattle (Vollman and Vollman 1942 rabbit (Donnet *et al* 1960 a) long tailed monkey rat and guinea pig (Donnet *et al* 1960 b) Recently cyclic variations have been demonstrated in some sexual functions of bulls and male rabbits (Dogget 1956 Kihlstrom 1958 1962 1963 Degerman and Kihlstrom 1961 Kihlstrom and Degerman 1962) Some facts indicate that in the rabbit the male sexual cycle like that of the female is hormonally regulated (Kihlstrom and Degerman 1962) Furthermore in this animal the male cycle is of the same duration 4—6 days as the estrus cycle of the doe (Myers and Poole 1962) Therefore it seems to be of interest to study whether or not a thermal cycle connected with the sexual functions exists also in males

Material and methods

Twenty five normal male rabbits all sexually mature and ranging in age from 8 to 10 months have been used for the experiments The animals were separately caged but could see each other as well as female animals. The temperature of the room was thermostatically regulated The feeding and care of the animals were carried out daily between 9 and 11 a m The body temperature was recorded daily using a calibrated electric universal thermometer (type TE 3 Ellab A S Copenhagen with a reading accuracy of ± 0 0 C Temperature recordings were

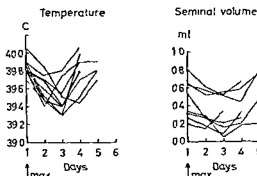


Fig 1 All cycles obtained from animal no 191 during March have been superimposed first maximum in the cycles placed on day number one

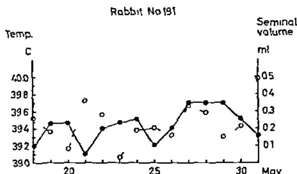


Fig 2 The daily recordings of body temperatures (solid line) and seminal volumes (broken line) obtained from animal no 191 from May 18 to May 31

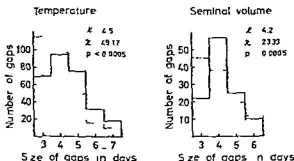


Fig 3 The observed distribution (solid lines) of the sizes of the gaps between minima compared with the theoretical distribution (broken lines) to be found in a series of random numbers

performed by inserting the applicator (type RK 2) 9 cm through the anus These measurements were carried out between 8 and 10 a m — a time when the body temperature in preliminary experiment was found to be at a minimum

Nine animals were studied from the beginning of February until the end of May Possibly existing connections between body temperature and sexual activity were studied by observing the volumes of ejaculates daily yielded. Semen was collected at a fixed time of the day by means of an artificial vagina and discharged into small test tubes the volumes of which up to a

TABLE I Statistically significant cyclic variations in body temperature

Rabbit no	Length (days)	Test for frequency of maxima t	f	p<	Comparison between mean values from columns with the highest numbers of maxima and minima respectively t	f	p<
257	8	1.79	86	0.05	2.64	22	0.01
198	9	2.09	82	0.025	2.04	16	0.05
191	7	2.73	129	0.005	2.02	28	0.05
190	8	3.11	121	0.005	3.50	26	0.001
158	4	3.00	95	0.005	2.80	37	0.005
157	8	2.16	69	0.025	2.51	16	0.025
156	7	1.98	86	0.05	2.47	20	0.025
155	3	2.41	90	0.01	2.23	50	0.025
	6	2.09	88	0.025	2.50	24	0.01
154	No significant cyclic variation						

mark in the glass wall were known with great accuracy The volume of the semen was then measured by adding distilled from a burette up to this mark.

The remaining 16 animals were studied as to temperature during at least a fortnight

To test whether the data obtained are distributed at random or not we used a test for randomness in a series of numerical observations, elaborated by Kendrew and McKendrick (1937) In this test figures smaller than either one of their immediate neighbours in the series are defined as minimum numbers The interval between two such minima expressed in time units in our case days is called a gap both minima being included with the gap In series of random numbers the theoretical distribution of gaps of different sizes has been calculated by Kermack and McKendrick (1937) By means of χ analysis this theoretical distribution is then compared with the distribution found in the material to be tested A non random distribution obtained by means of this test is a good indication of cyclic variations Using this method the only relevant fact is the interval between the minima the sizes of these numbers being irrelevant This means that very small and occasionally occurring minima are of the same importance as more pronounced and regularly occurring minimum values which in turn gives a surplus of small gaps

More detailed information concerning the cyclic variations has been obtained using the data from the animals studied daily from February to May The collected figures have been arranged in chronological order with a constant time interval of one day Part of a series thus obtained is diagrammatically illustrated in Fig 2 Each series was then rearranged in horizontal rows of identical length one below the other This arrangement was repeated the number of figures in each row varying from 3 to 20 (Cf Worthing and Geffner 1944 p 296) and the mean value of each vertical column calculated In the series all maxima and all minima irrespective of their deviations from the adjacent values were marked and the maximum numbers used for the further statistical treatment Unless the collected data express cyclic variations these values will be distributed at random among the vertical columns obtained by the procedure described above *However a cyclic variation of a given length appears as an* accumulation of the majority of the maxima in one and the same column This happens when the horizontal row has a length approaching that of the cycle or a multiple of the cycle The probability of a given frequency of maxima in a vertical column to be statistically different

TABLE II Changes in seminal volumes following upon maxima and minima in temperature

	Seminal volume			
	No of increases		No of decreases	
Temperature	Observed	Expected	Observed	Expected
Maximum	47	60	80	67
Minimum	67	65	69	1

$\chi^2 = 4.99$ $p < 0.05$

from that expected if the maximum values of the whole series were distributed at random among the columns has been tested as described earlier (Kihlstrom 1962 1963) However in this test as in that described above small maxima and minima are of the same importance as those deviating markedly from their adjacent values In order to overcome this disadvantage a statistically significant accumulation of maxima in a column has not been looked upon as proving a cyclic variation unless the mean value calculated in this column differs significantly from that calculated in the column having the greatest number of minima The connections between temperature recordings and seminal volumes have been tested by means of chi-square analysis

Results

Examples of the daily temperature and volume recordings are diagrammatically represented in Fig 1 and 2

Body temperature The mean of all temperature data is 39 34 ± 0 001 (S E) As seen from Fig 3 the distribution of the lengths of the gaps between temperature minima when data from all animals are pooled is far from random ($\chi^2 = 49.17$ df = 4 $p < 0.0005$) which strongly indicates a cyclic variation These gaps have a mean length of 4 5 days When applying the test for frequency of maxima described above eight of nine animals studied revealed a statistically significant cyclic variation the cycles varying in length from 3 to 9 days with a mean value of 7 days See Table I)

Seminal volumes The distribution of the gaps between the minima in seminal volumes is given in Fig 3 This distribution too is far from random ($\chi^2 = 23.33$ df = 3 $p < 0.0005$) The mean length of the gaps is 4 2 days The cyclic variation in the seminal volumes have been described earlier (Degerman and Kihlstrom 1961)

A maximum in temperature is significantly more frequently followed by decreasing volumes than is the case concerning a minimum in temperature (χ^2 4 99 $p < 0.05$ See Table II)

Discussion

With a very high degree of probability the body temperature of male rabbits varies cyclically This variation shows a statistical coincidence in phase with the cycles of the daily seminal volumes Besides, the durations of the cycles are in good agreement with the length earlier discovered on studying the volumes of ejaculates (Kihlstrom 1958

Degerman and Kihlstrom 1961) the sexual drive (Degerman and Kihlstrom 1961) production and density of sperm cells (Doggett 1956) and the occurrence of gels in the seminal fluid (Kihlstrom and Degerman 1962) It may be mentioned that the length of the female estrus cycle in domesticated rabbits is 4—6 days (Myers and Poole 1962)

The mean length of the gaps between minima in body temperature is 4 5 days As discussed above random variations will give a surplus of small gaps This fact probably explains the greater mean value 7 days of the significant cycles found using the test for frequency of maxima However it is also possible that a cycle having a length of about 4 days is co-existent with a longer one (See Table I rabbit number 155)

As the cyclic variations of the different animals caged in the same room are not synchronous environmental causes of the phenomena must be excluded

The variation in body temperature after castrating the animals is under observation and the results will be published elsewhere

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Acute Effects of Prolonged, Heavy Exercise on the Concentration of Plasma Lipids and Lipoproteins in Man

By

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Abstract

Carlson L A and F Mossfeldt *Acute effects of prolonged heavy exercise on the concentration of plasma lipids and lipoproteins in man.* Acta physiol scand 1964 62 51—59 — The concentration of triglycerides cholesterol and phospholipids in plasma and in ultracentrifugally separated plasma lipoproteins was studied in normal persons during participation in 1962 and 1963 in a yearly skiracing The skiing time was around 8—9 hours In the group studied in 1962 as well as in the 1963 group there was a significant fall in the concentration of triglycerides and phospholipids in plasma When the 1962 group was studied during ordinary activities with identical caloric intake and at identical times as during the skiing no significant changes were found in the plasma lipids The most pronounced decreases of the plasma lipids was in the triglyceride fraction and this decrease was directly and highly significantly correlated to the fasting triglyceride concentration About three quarters of the decrease in triglyceride concentration was due to a decrease in the amount of triglycerides in the very low density lipoproteins The triglyceride concentration in the low and the high density lipoprotein classes also decreased The decrease of triglycerides was directly correlated to the fasting level in each lipoprotein class No significant changes were observed in the cholesterol content of any of the lipoproteins. The phospholipid concentration however decreased in all three lipoprotein classes The most pronounced decrease of phospholipids was found in the high density lipoproteins Mechanism(s) for these changes in the concentration of the plasma lipids and lipoproteins during prolonged, heavy exercise were discussed.

Previous studies in man have shown that the concentration of the free fatty acids (FFA) of plasma are characteristically changed during exercise in the fasting state Early during exercise the arterial concentration of FFA decreases (Carlson and Pernow 1959 Friedberg et al 1960 Carlson and Pernow 1961 Bruce, Cobb and Williams 1961) due to an increased fractional turnover rate (Carlson and Pernow 1961) After about 15 min of exercise the FFA level starts to increase (Basu Passmore and Strong 1960 Carlson and Pernow 1961 Friedberg et al 1963 Havel Naimark and Borchgrevink 1963) due to an increased mobilisation (Friedberg et al 1963 Havel et al 1963)

These events can be looked upon as a purposeful mechanism to supply energy in the form of fatty acids to the working muscles. If other fatty acids of plasma, i. e. the different forms of esterified fatty acids, could contribute to the energy needs during exercise in man is not known. Since we had observed that no changes occurred in the concentration of plasma cholesterol, phospholipids and triglycerides during exercise at moderate work loads for up to two hours (Carlson and Pernow, unpublished results) it was considered to be of interest to study these plasma lipids during prolonged heavy exercise. In this paper data are given on changes in the plasma lipids and lipoproteins during participation in skiracing for about eight to nine hours.

Material and methods

Material. The studies were done in 1962 and 1963 during "Vasaloppet", which is a skiracing occurring every year in March in Dalecarlia, Sweden. The racing consists of skiing 85 km in hilly terrain with start at one place (Salen) and the winning post at another place (Mora).

The persons studied were all in good health. All subjects had taken part in physical training during 5 months before the experiment. The training consisted of gymnastics indoors the hospital. The exercise took special aim to train the circulatory capacity and the muscles that are specially used in skiing. The last two months before the experiment the training was recompleted with skiing. On the average 300 km was run on skis during this time before the start in Vasaloppet.

The material studied in 1962 comprised 9 men: 6 doctors, one dentist and 2 managers. Twenty persons took part in "Vasaloppet" 1963: 14 doctors, one photographer, one insurance man, one shipbroker, one teacher and 2 managers. The 1962 group was also studied during their ordinary activities about 3 months after "Vasaloppet". During that study they consumed identical amounts of food and liquids at the same time as during "Vasaloppet", and blood samples were also taken at identical times.

Venous blood samples were taken at 6 a. m. after fasting over night. The subjects then consumed their breakfast. In 1962 the subjects had their own free choice for breakfast. In 1963, however, the breakfast was designed to contain as little fat as possible. The skirace then started at 7.45 a. m. Stations, where water containing 30 per cent sugar was given, were situated 21 km, 25 km, 28 km, 33 km, 41 km, 46 km, 54 km, 60 km, 72 km, and 79 km after the start. The heart rate was examined after skiing 21 km, 28 km, 41 km, 54 km, 63 km, 72 km and immediately after finished skiing and the mean heart rate for each subject was calculated from these occasions. The surface for skiing was easier during 1962 than 1963. Within 5—10 min after the subjects arrived to the winning post a second venous blood sample was drawn. The blood samples were heparinised, plasma separated off by centrifugation and stored at 5—10° C for about 24 hours before being processed at the laboratory.

Lipid analysis. The plasma lipids were determined as described previously (Carlson 1960, Carlson 1963). Plasma protein analysis were made with the Biuret method and all lipid values corrected for any changes in plasma protein concentrations.

Lipoprotein analysis. The plasma lipoproteins were separated in principle according to Bragdon, Havel and Boyle (1955). Four ml of plasma was added to the tubes of the 40.3 rotor of the Spinco Model L preparative ultracentrifuge and diluted with 0.15 M NaCl. After centrifugation at 40 000 rpm for 18 hours the tubes were sliced and the top fraction harvested quantitatively and made up to 3 ml with saline. This fraction is called *very low density lipoproteins*. The bottom fraction was aspirated and recovered quantitatively from the sliced tubes and made up to 5 ml with 0.15 M NaCl. Of this 4.5 ml was added to a new tube and one ml of a NaCl—KBr solution with a density of 1.3197 added. The tubes were then filled up with a NaCl—KBr solution of density 1.063, capped, mixed and centrifuged as above. After centrifugation the tubes were sliced, the top fraction harvested quantitatively and made up to 3 ml with saline. This fraction is called *low density lipoproteins*. The bottom fraction was transferred quantitatively to a 5 ml volumetric flask with saline and is called *high density lipoproteins*. *One ml of these fractions* was extracted with chloroform methanol for determination of triglycerides (Carlson 1963) and 1.25 ml extracted with ethanol acetone for determination of cholesterol — *according to* Sperry-Webb — and phospholipids as described previously (Carlson 1960). The entire procedure gives a recovery of all lipid fractions between 95—105 per cent.

TABLE I Age, physical working capacity, caloric consumption, skiing time and heart rate in the 1962 and 1963 group

Material	Age[1] (yrs)	Mean physical working capacity (kpm/min)	Caloric consumption: Total calories (kcal)	Calories from carbohydrate (kcal)	Calories from fat (kcal)	Mean skiing time for Vasa loppet (hrs)	Mean heart rate during skiing (/min)
1962 group	36 [illegible]4–5[illegible]	1 460	2 8[illegible]0 ± 1 0[illegible]0	[illegible] 0[illegible]0 ± 1 0[illegible]0	3[illegible]0 ± 90	8 67	144
1963 group	34 22–53	1 5[illegible]0	4 180 ± [illegible]0	3 960 ± 700	4[illegible] ± 20	9 [illegible]9	1[illegible]4

[1] Mean and range
Defined as physical working capacity at a heart rate of 1 0/min under standardised conditions on a bicycle ergometer (Holmgren 1956)
Mean value ± standard deviation

TABLE II The effect of skiing on plasma lipid levels as compared to the plasma lipid levels during ordinary activities in 9 normal men (196[illegible] series) Each person consumed identical amounts of food and liquid during the two different studies and food as well as the blood samples were taken at identical times in the two different studies

	Cholesterol (mg/100 ml) Fasting	After	Phospolipids (mg/100 ml) Fasting	After	Triglycerides mmoles l Fasting	After
During skiing						
Mean ± S D	2[illegible]8 ± 5[illegible]	199 ± [illegible]	[illegible]66 ± 43	[illegible]30 ± 44	1 [illegible] ± 0 [illegible]0	0 7[illegible] ± 0 [illegible]
Ind diff	29 ± 10		37 ± 8		0 [illegible]1 ± 0 [illegible]0	
P	0 05		0 01		0 0[illegible]	
During ordinary activities						
Mean ± S D	221 ± [illegible]5	3[illegible] ± 56	[illegible]6 ± [illegible]3	[illegible]6 ± 48	1 33 ± 0 [illegible]3	1 [illegible]4 ± 0 [illegible]
Ind diff	−11 ± [illegible]		0 ± 9		0 10 ± 0 1	
P	0 05		0 0[illegible]		0 0[illegible]	

Calculated by subtracting the value after from fasting for each subject Mean value ± standard error of the mean.
Statistical significance of the individual differences

TABLE III The effect of skiing on plasma lipid levels in 20 normal men (1963 series)

	Cholesterol (mg/100 ml)		Phospholipids (mg/100 ml)		Triglycerides (mmoles/l)	
	Fasting	After	Fasting	After	Fasting	After
Mean ± S.D	20[illegible] ± 36	196 ± 36	250 ± 31	224 ± 30	1.2[illegible] ± 0.49	0.78 ± 0.31
Ind. diff.[1]	6 ± 4		27 ± 5		0.44 ± 0.07	
P[2]	> 0.05		< 0.001		< 0.001	

[1] and [2] See Table II

TABLE IV The effect of skiing on the concentration and composition of the plasma lipoproteins in 10 men (1965 series)

	Cholesterol (mg/100 ml)		Phospholipids (mg/100 ml)		Triglycerides (mmoles/l)		Ratio Cholesterol/Phospholipids	
	Fasting	After	Fasting	After	Fasting	After	Fasting	After
Very Low density lipoproteins								
Mean ± S.D	14 ± 10	8 ± 6	18 ± 13	10 ± 9	0.6[illegible] ± 0.40	0.26 ± 0.19	0.78 ± 0.19	0.81 ± 0.23
Ind. diff.	4.8 ± 2.4		7.2 ± 2.9		0.37 ± 0.09		0.03 ± 0.05	
P[2]	> 0.05		< 0.05		< 0.01		> 0.05	
Low density lipoproteins								
Mean ± S.D	12[illegible] ± 25	118 ± 2[illegible]	87 ± 16	7[illegible] ± 2[illegible]	0.40 ± 0.13	0.30 ± 0.09	1.42 ± 0.06	1.4[illegible] ± 0.10
Ind. diff.	4.[illegible] ± 6.0		7.9 ± 2.9		0.0[illegible]6 ± 0.031		0.08 ± 0.04	
P	> 0.05		0.05		< 0.05		> 0.05	
High density lipoproteins								
Mean ± S.D	63 ± 9	65 ± 13	148 ± 16	134 ± 21	0.19 ± 0.06	0.15 ± 0.03	0.4[illegible] ± 0.03	0.49 ± 0.03
Ind. diff.[1]	−1.9 ± 1.4		14.2 ± [illegible]7		0.04 ± 0.01		−0.06 ± 0.01	
P	> 0.05		< 0.01		< 0.05		< 0.001	

[1] and [2] See Table II

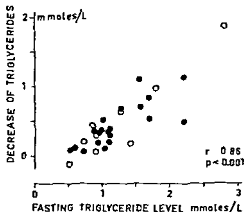


Fig 1 Relationship between the fasting concentration of triglycerides in plasma and the decrease of triglyceride concentration in plasma during Vasaloppet ○ 1962 group ● 1963 group

Results

Some data for the two groups are given in Table I The caloric consumption the skiing time and the heart rate were all increased during Vasaloppet 1963 These findings are probably due to the fact that the surface for skiing was heavier in 1963 than in 1962 Approximately half of the calories from carbohydrates were consumed during the skiing in the form of water containing sugar

Plasma lipids during skiing and during ordinary activities in the 1962 group

The plasma concentration of cholesterol, phospholipids and triglycerides before and after the two different activities are given in Table II The fasting levels were the same at the two occasions During skiing there was a significant fall in all plasma lipids while no significant changes occurred during ordinary activities

Plasma lipids during skiing in the 1963 group

The plasma lipids before and after skiing in the 1963 series are given in Table III There was a significant fall in the concentration of phospholipids and triglycerides during skiing It can also be seen that the fasting lipid levels were similar to the levels in the 1962 group Furthermore the decrease in the concentration of triglycerides and phospholipids was of the same magnitude in the two groups

Plasma lipoprotein composition during skiing

The lipid composition of the three lipoprotein classes before and after skiing is given in Table IV In the *very low density lipoproteins* there was a significant fall in the concentration of phospholipids and triglycerides The phospholipids and triglycerides decreased significantly also in the *low density lipoprotein* fraction The fall in triglycerides in this fraction was however only one quarter of the fall in the very low density lipoproteins In the *high density lipoproteins* the concentration of phospholipids and triglycerides were significantly reduced The ratio cholesterol to phospholipids increased significantly in this lipoprotein fraction

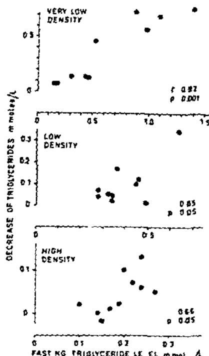


Fig 2 Relationship between the fasting concentration of triglycerides in three different plasma lipoprotein classes (very low density lipoproteins low density lipoproteins and high density lipoproteins) and the decrease of triglyceride concentration in these different lipoprotein classes

Relationship between the fasting lipid levels and the changes induced during skiing

No correlation was found between the fasting levels of plasma cholesterol or phospholipids and the changes in these fractions during exercise The relationship between the fasting triglyceride concentration and the decrease in this fraction during exercise is given in Fig 1 for the 1962 and 1963 groups There is a linear and highly significant relationship between the decrease in triglyceride concentration and the fasting triglyceride level It can also be seen that this relationship is very similar for the 1962 and 1963 series

A similar relationship exists in the separated very low density lipoprotein class (Fig 2) There is also a tendency for the same relationship in the other two lipoprotein classes (Fig 2) The higher the fasting triglyceride concentration was the greater was the fall in this concentration in all three lipoprotein classes

Discussion

The most important findings in this study was that the concentration of triglycerides and phospholipids in plasma was reduced during prolonged heavy exercise This immediately raises the questions if this effect was due to the exercise or to the heavy carbohydrate load and if the effect was on the endogenous or on exogenous plasma lipids

Carbohydrates affect the plasma triglyceride levels acutely A load of 400 g glucose given to fasting persons thus causes a significant fall in the concentration of triglycerides (Havel 1957) However when we studied the plasma lipids during ordinary activity

when the subjects consumed the same kind and amount of food at identical times as during "Vasaloppet" no significant change in the concentration of any plasma lipid fraction was observed. This indicates that the changes observed during "Vasaloppet" was due to the exercise and not to the carbohydrate load. Furthermore there was no relationship between the intake of carbohydrates and the decrease of triglycerides in either the 1962 or the 1963 group.

Exercise has been shown to decrease the turbidity of alimentary lipemia (Cohen and Goldberg 1960) as well as to reduce the increase in plasma triglyceride concentration normally seen after a fatty meal (Nikkila and Konttinen 1962). If this is due to an alteration in the absorption of lipids from the intestine during exercise or to an increased removal rate of chylomicrons — transporting the dietary fatty acids — is not known. The changes we observed in triglycerides during "Vasaloppet" is with all probability an effect on endogenous triglycerides. First, the triglyceride concentration decreased from the fasting level. Secondly, in the 1963 group the fat consumption was on the average only 5 g.

The major part — about three quarters — of the decrease in triglyceride concentration was due to a decrease of triglycerides contained in the very low density lipoproteins. The triglyceride concentration in this fraction decreased more than 50 per cent. In the low and the high density lipoproteins the triglyceride content decreased about 25 per cent but due to the relatively small amounts of triglycerides present in these fractions these decreases together contributed only to about one quarter of the total decrease of the plasma triglycerides.

Of the other lipid components of the lipoproteins the cholesterol content was not changed significantly while the phospholipid concentration decreased in all three classes and most so in the high density lipoproteins. However, on a percentage basis the drop in phospholipids was smaller than the drop in triglycerides in all lipoprotein classes (40, 9 and 10 per cent respectively for phospholipids versus 59, 24 and 23 per cent for triglycerides). Prolonged exercise thus influenced the different lipid components of the lipoproteins unequally. This suggests that the effect exerted by exercise was on a part of and not on the entire lipoprotein molecule.

The two possibilities for the cause of the decrease of plasma triglycerides during exercise — a decreased influx to plasma or/and an increased efflux from plasma — will be briefly commented upon in relation to current concepts of the transport of triglyceride fatty acids in plasma. Since at least part of the endogenous plasma triglycerides are derived from the liver (Harper, Neal and Hlavacek 1953, Stein and Shapiro 1959, Borgström and Olivecrona 1961, Havel and Goldfien 1961, Carlson and Ekelund 1963) a decreased influx of triglycerides to plasma could be caused by a decreased delivery of triglycerides from the liver to the plasma. This might occur as a result of decreased hepatic uptake of plasma FFA since FFA is an important precursor to the liver and subsequently the plasma triglycerides (Stein and Shapiro 1959, Borgström and Olivecrona 1961, Havel and Goldfien 1961, Havel, Felts and Van Duyne 1962). During exercise the mobilisation of FFA from adipose tissue is actually increased (Friedberg et al. 1963, Havel et al. 1963) but data are available suggesting that the percentage flux of FFA to the muscles at the same time is increased (Carlson and Pernow 1959, Carlson and Pernow 1961). It is also known that the hepatic blood flow is considerably reduced during heavy exercise (Wade and Bishop 1962). This suggests that the uptake of FFA in the liver may be decreased during exercise which could result in a decreased formation of triglycerides in the liver. The rapid equilibration between the hepatic and plasma

triglyceride pools seen in rabbits (Havel et al 1962) and in man (Carlson and Ekelund 1963) makes it probable that a decrease of the hepatic triglyceride pools should be reflected in a decrease of the plasma triglyceride pools In support of this hypothesis it has in fact been shown that there is a lesser incorporation during exercise than at rest of injected labeled FFA into the plasma triglycerides of man (Havel et al 1964)

An increased efflux of triglycerides from plasma during exercise could be due to increased uptake of triglyceride fatty acids in peripheral organs such as the working muscles In comparison with the flux of FFA through plasma the transport of plasma triglyceride fatty acids to the periphery is low in the fasting state in resting man (Carlson and Ekelund 1963) It has however been reported that in the post prandial state the beating heart takes up more esterified fatty acids than FFA (Ballard et al 1957) Furthermore during *in vitro* perfusion of the rabbit heart the concentration in the perfusate of triglycerides contained in the very low density lipoproteins also decreases (Gousios Felts and Havel 1963) An uptake of triglyceride fatty acids thus occurs in peripheral organs This uptake of triglyceride fatty acids might be regulated by the action of lipoprotein lipase (Robinson and French 1960) Since exercise increases the lipoprotein lipase activity in the heart of rats (Nikkila Torsti and Penttila 1963) a possible mechanism for increased efflux of plasma triglyceride fatty acids to the periphery during exercise appears to exist

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Comparative Investigation of the Calorigenic and Lactic Acid Stimulating Effects of Isoprenaline and Adrenaline in Experiments on Rabbits

By

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Abstract

Lundholm L and N Svedmyr *Comparative investigation of the calorigenic and lactic acid stimulating effects of isoprenaline and adrenaline in experiments on rabbits* Acta physiol scand 1964 62 60—67 — The stimulating effects of DL-isoprenaline and L-adrenaline on oxygen consumption and lactic acid production were compared in experiments on rabbits In doses which produced a submaximal response DL-isoprenaline was for both effects 4—5 times as potent a stimulant as L adrenaline Although the calorigenic action of isoprenaline was largely referable to increased lactic acid oxidation the fact that isoprenaline in the doses tested markedly stimulated the heart rate suggests that an increased cardiac activity contributed to the calorigenic effect Intra arterial infusion of isoprenaline into the brachial artery increased the elimination of lactic acid from skeletal muscle while commensurately augmenting the blood flow It seems probable that the vasodilator effect of isoprenaline in the rabbit stemmed at least in part from increased lactic acid production in the skeletal muscle — The relations for doses of L-isoprenaline L adrenaline and L noradrenaline which produced equal lactic acid producing and calorigenic effects in the rabbit were 1 9 120

Previous investigations have revealed a close correlation between adrenaline s stimulation of lactic acid production and its calorigenic and vasodilator effects (Lundholm 1949 1956 1957 1958 Lundholm and Mohme Lundholm 1960) In the present study we sought to determine whether a similar correlation exists in the case of isoprenaline Isoprenaline stimulates oxygen consumption in the rat (Waterman 1949) and in man (Cobbold Ginsburg and Paton 1960) Its effect upon carbohydrate metabolism however seems to be more variable In experiments on rats isoprenaline raised the lactic acid content of the blood but produced no hyperglycemic effect (Ellis and Anderson 1953) A hyperglycemic action was however demonstrated in rabbits (Ellis 1956) In terms of a glycogenolytic action on isolated rat diaphragm isoprenaline was ten times as potent as adrenaline (Ellis Davies and Anderson 1955 Kennedy and

Ellis 1963) while in experiments on dog s heart it was ten times as potent as adrenaline in the activation of phosphorylase *a* (Mayer and Moran 1960) On the other hand Cobbold Ginsburg and Paton (1960) in experiments on humans found no augmentation of lactic acid production after administration of isoprenaline in doses which had appreciable vasodilator and stimulatory effects on the metabolism

In view of these findings we resolved to study the question of whether isoprenaline — in doses producing a calorigenic effect — also elevated the lactic acid production and to compare the results with those referable to adrenaline In the rabbit experiments described below we observed a distinct correlation between the lactic acid stimulating and the calorigenic effects of isoprenaline

Isoprenaline in the doses tested increased the heart rate appreciably — an effect not found with equivalent doses of adrenaline The augmented cardiac activity could presumably have helped to stimulate the oxygen consumption The calorigenic effects of isoprenaline and adrenaline showed however no significant difference that could be ascribed to isoprenaline s cardioaccelerator action It would seem nevertheless that this powerful cardioaccelerator effect of isoprenaline is in humans a factor which probably contributes to its calorigenic action (Lundholm and Svedmyr to be published)

Methods

Oxygen Consumpti n — The O consumpt on was determ ned in rabbits hich had fasted for 15—18 hours. Under local anesthesia one cannula was introduced into the central artery of an ear and a second cannula into the marg nal e n of the contralateral ear Following i v injection of 5 per cent heparin solut on the animal was placed in a metabolism apparatus for determinat on of oxygen consumpt on as described previously (Lundholm 1949) Fine rubber tubes were passed from the cannulae through the l d of the apparatus so that blood samples could be drawn f om the artery or solutions nfused into the vein without disturbing the animal After about 60 min hen the animal had become accustomed to the conditions and the blood level of lactic acid measured a basal value (e < 10 mg per cent) the basal oxygen consumption was determined fo 60 min in 10-m n periods and blood samples were taken for determ nat on of the basal lactic acid content 10 min before the start of infus on DL sop enaline sulphate or L-adrenaline hyd ochloride was thereafter infused i v over a 60-min period at the rate of 0 3 ml min and in a dose of 0 05 and 0 5 μg/kg/min calculated as base The drugs we e dissolved in sterile pyrogen free 0 9 per cent sodium chloride solution to which 0 13 pe cent asco bic ac d had been added to p event oxidat on of the sympathomimetic amines Oxygen consumption was measured for 180 min as from the start of infusion while the lactic acid content of the blood was assayed 20 40 60 90 120 and 180 min after the start of infusion

Heart Rate a d Blood Pressu e — In a ser es of 6 animals we recorded the effects on the heart rate of DL-isoprenal ne and L-adrenaline infused i v for a 20-min period in doses of 0 05 and 0 5 μg kg/m n Heart rate was recorded by a tachycardiograph on Grass instruments In an add t onal 4 experiments we determined the effects of adrenaline and isoprenal ne on the blood pressu e With the animal under Ev pan anesthesia and local anesthesia with lidoca ne we cannulated the r ght carot d and recorded the blood pressure by Statham p essure transduc r on Grass nstruments after heparini ng the rabbit w th 1 ml of 5 per cent heparin solu tion Approximately 4 hours after preparation hen the animal had recovered from the general anesthesia isoprenaline or adrenal ne was infused and the effects on blood pressur and heart rate were recorded for 20 min

B ood Flow — For i a infus on of isoprenaline the rabb ts were anesthetized with urethane 1 4 g/kg The trachea was cannulated and the an mal breathed into a small Krogh spirometer with 100 O This procedure was des gned to reduce the effect of anesthesia on the blood level of l ctic acid (Lundholm 1957) but failed to achieve its purpose since the basal lactic acid content in these experiments was high — approx mately 50 mg per cent. A *T* cannula was inserted into the right brachial artery the circulating blood passing through its horizontal limb

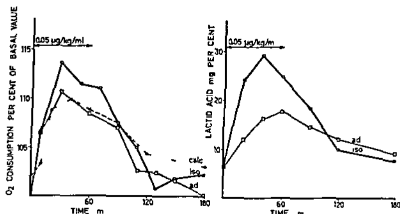


Fig 1 Effects of 0 05 µg/kg/min DL-isoprenaline ($n = 8$) and 0 05 µg/kg/min L adrenaline ($n = 6$) on oxygen consumption and lactic acid content of the blood The drugs were infused for 60 min i v Oxygen consumption in percent of the basal value was measured for 60 min prior to infusion The calculated curve represents the rise in oxygen consumption attributable to increased lactic acid oxidation in the experiments with isoprenaline

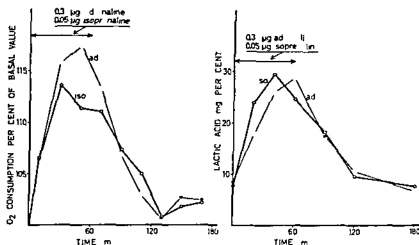


Fig 2 Effects of 0 05 µg/kg/min DL-isoprenaline and 0 3 µg/kg/min L adrenaline ($n = 6$) on oxygen consumption and lactic acid content of the blood

During the experiment DL-isoprenaline 0 1 µg per min in 0 1 ml of 6 per cent dextran solution was infused through the vertical limb Blood flow was registered by drop recorder via a cannula in the brachial vein The blood was then collected for 10 min in a measuring chamber from which samples were taken for lactic acid determination in venous blood The blood was returned to the animal via a cannula in the jugular vein Before the experiment was begun 1 ml of 5 per cent heparin soution and 15—20 ml of 6 per cent dextran were administered i v

Lactic acid content was determined *ad modum* Friedmann and Graeser (1933) in 1 5 ml blood precipitated with 15 ml 10 per cent trichloracetic acid Duplicate determinations were made on the extract

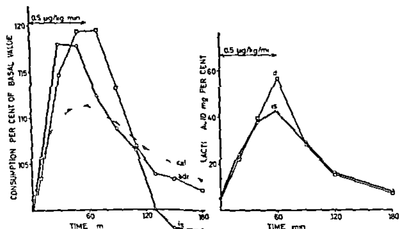


Fig 3 Effects of 0 5 μg/kg/min DL-isoprenaline (n = 5) and L-adrenaline (n = 9) on oxygen consumption and lactic acid content of the blood See also legend to Fig 1

TABLE I Effects of DL-isoprenaline and L-adrenaline on oxygen consumption in unanesthetized rabbits The drugs were infused for 60 min i v Oxygen consumption was measured for 60 min prior to infusion and for 120 min after infusion was begun. Oxygen consumption during and after infusion was calculated in percent of the basal value

Drug	DL-isoprenaline		L-adrenaline		
Dose (µg/kg/min)	0 05	0 5	0 05	0 3	0 5
Mean increas ( )	109 4±2 9	112 5±2 5	106 6±1 7	110 8±2 5	114 0±2 ?
No of tests	8	5	6	6	9
P	0 01	<0 01	<0 01	<0 01	<0 001

Results

Oxygen Consumption — The effects of DL-isoprenaline (base) 0 05 μg (n = 8) and 0 5 μg (n = 5) per kg per min on oxygen consumption and lactic acid content of the blood will be evident from Fig 1—3 and Tabl I where the effects are also compared with those of L-adrenaline (base) 0 05 μg (n = 6) 0 3 μg (n = 6) and 0 5 μg (n = 9) per kg per min In the dose of 0 05 μg isoprenaline had an appreciably greater effect on both oxygen consumption and lactic acid content than had adrenaline At the end of 40 min infusion the blood level of lactic acid for example was 29 4 ± 6 1 mg per cent in the experiments with isoprenaline but only 16 2 ± 2 3 mg per cent in those with adrenaline The difference of 13 3 ± 6 0 was statistically probable (P < 0 05 n = 14) On the other hand 0 05 μg isoprenaline had approximately the same effect on both oxygen consumption and lactic acid content as did 0 3 μg adrenaline (Fig 2) Adrenaline in a dose of 0 5 μg appeared to have somewhat stronger effects than did 0 5 μg isoprenaline though the difference was not statistically significant

Fig 4 shows the dose response curves for the effects of isoprenaline and adrenaline on the oxygen consumption — It should be noted that 0 5 μg adrenaline has a maximal

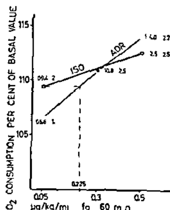


Fig 4 Correlation between dose of DL-isoprenaline and L-adrenaline and mean effect on oxygen consumption 0—120 min after start of drug infusion 0 05 µg/kg/min of DL isoprenaline was equivalent to 0 225 µg L adrenaline

effect (Lundholm 1949) — and the same may well be true of isoprenaline This could account for the fact that the curves converge towards 0 5 µg/kg/min — We calculated graphically from the curve the dose of adrenaline that was equivalent in effect to 0 05 µg isoprenaline and obtained a value of 0 225 µg Isoprenaline was administered as racemic salt and since its action was largely attributable to the L-isomer (Dornhorst and Herxheimer 1958) it may be assumed that L-isoprenaline was approximately 9 times as potent as L adrenaline in stimulating the oxygen consumption The same assumption applies to the stimulation of lactic acid production (Fig 2) In previous experiments on rabbits (Lundholm 1950) L noradrenaline was found to be about 13 times weaker than L-adrenaline in its stimulatory effect on oxygen consumption and lactic acid production The relations for doses of isoprenaline adrenaline and noradrenaline which produced equal effects were therefore 1 9 120

Heart Rate and Blood Pressure — On infusion of DL isoprenaline or L-adrenaline 0 05 or 0 5 µg/kg/min in unanesthetized rabbits the effects on blood pressure and heart rate reached a maximum after 5—10 min and remained at that level throughout the infusion Isoprenaline in each of the two doses increased the heart rate appreciably (Table II) while the larger dose conspicuosly depressed the systolic and diastolic blood pressures Adrenaline had considerably milder effects The 0 5 µg dose was associated with a slight reduction of the heart rate and some increase of the pulse pressure in consequence of a decline in the diastolic and a rise in the systolic pressure Earlier experiments had shown however that adrenaline in similar circumstances increased the cardiac output by about 50 per cent via augmentation of the stroke volume without substantially influencing the heart rate or blood pressure (Lundholm 1958) The negative findings referable to adrenaline cannot therefore be taken as proof that adrenaline in these circumstances did not stimulate the heart activity

Effect of Intra arterial Infusion of Isoprenaline on Blood Flow and Lactic Acid Elimination from Skeletal Muscle — In this experiment we studied the effects of isoprenaline on the blood flow and the lactic acid production in skeletal muscle and sought to rule out the possibility that the latter effect was produced reflexly via liberation of endogenous adrenaline or noradrenaline from the suprarenals Isoprenaline was infused into the area supplied by the right brachial artery Its effect on the lactic acid production was determined by calculating the elimination of lactic acid from the product of the arterio-

TABLE II Effects of DL-isoprenaline and L adrenaline on heart rate and blood pressure in experiments on unanesthetized rabbits The drugs were infused i v for 20 min In the experiments with isoprenaline the values were recorded after 10 min infusion in those with adrenaline after 5 min n = number of tests P = probability that the effect was due to chance

Drug and dose	Heart rate Basal	Heart rate Change per cent after drug	Blood pressure mm Hg Basal	Blood pressure mm Hg After drug
DL-isoprenaline				
0 05 µg/kg/min	247	119 ± 3 8	146/105	145/101
	$n = 9$	$P < 0\ 001$	$n = 4$	
0 5 µg/kg/min	237	146 ± 6 7	141/107	129/86
	$n = 7$	$P < 0\ 001$	$n = 4$	
L-adrenaline				
0 05 µg/kg/min	265	102 ± 1 0	143/103	145/100
	$n = 9$		$n = 4$	
0 5 µg/kg/min	253	95 ± 1 4	146/106	149/103
	$n = 7$	$P < 0\ 01$	$n = 4$	

venous lactic acid difference and the blood flow It follows that determination of lactic acid production was not fully quantitative since it did not include the amount retained in the muscle On the other hand the lactic acid elimination was also determined for 10 min after termination of the infusion and during that interval the greater part at least of the lactic acid produced in the muscle may be assumed to have been eliminated in the blood (*cf* Lundholm 1956)

DL isoprenaline was infused in a dose of 0 1 µg/min for 10 min Its vasodilator effect was comparatively mild the blood flow increasing by a maximum of 100 per cent In some instances no vasodilator action was manifest and not even an increase of the dose to 1 µg/min produced an appreciable effect

The results may be seen in Fig 5 which gives the mean of 5 experiments in which the lactic acid elimination was determined for 10 min periods It is evident from Fig 5 that both blood flow and lactic acid elimination increased during the infusion of isoprenaline From an initial mean value of 12 6 ± 2 2 ml/10 min the blood flow rose by 30 ± 7 8 per cent (P < 0 01) while the lactic acid elimination rose from 0 38 ± 0 21 mg/10 min to 1 04 ± 0 25 mg/10 min the increase of 0 66 ± 0 16 mg/10 min being statistically significant (P < 0 02) In some experiments the blood flow and lactic acid elimination remained elevated during the post infusion period but the effects were not statistically significant A correlation between the lactic acid stimulating and the vasodilator effects of isoprenaline was apparent in these experiments

Previous investigations have shown that infusion of lactic acid has a vasodilator effect in experiments on rabbits (Lundholm 1958) It seems likely therefore that in

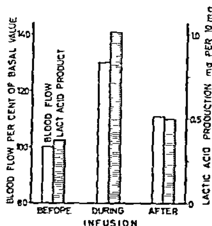


Fig 5 Effects of 0 1 μg/min isoprenaline on blood flow and lactic acid elimination in rabbit's foreleg Isoprenaline was infused for 10 min into the brachial artery blood flow and lactic acid elimination in brachial vein were measured for 10-min periods before, during and after the infusion. Mean lactic acid content of arterial blood was 48 2 ± 0 8 mg per cent. Lactic acid production mean of 5 determinations Blood flow mean of 10 determinations

the present experiments the lactic acid production of the skeletal muscle was to some extent, a factor in isoprenaline's vasodilator effect. On the other hand isoprenaline stimulates lactic acid production in isolated vascular muscle while relaxing the muscle (Lundholm and Mohme Lundholm, to be published) thus lactic acid production may in skeletal as well as in vascular muscle contribute to the vasodilator effect of isoprenaline. In experiments on humans isoprenaline appeared to have a vasodilator effect that could not be attributed entirely to increased lactic acid production in skeletal muscle (Lundholm and Svedmyr to be published)

Discussion

In previous experiments on rabbits it had been found that an increase in the lactic acid content of the blood was associated with stimulation of the oxygen consumption (Lundholm 1949 After i. v. infusion of L (+) lactate the following relation between oxygen consumption in percent of the basal value (Y) and the lactic acid content of the blood in mg per cent (X) was demonstrable $Y = 11\ 2 \log X - 93\ 2$ This relation tended to overestimate somewhat the effect of a low lactic acid content on the oxygen consumption but underestimated the effect of higher lactic acid concentrations We calculated, on the basis of this relation, the augmentation of oxygen consumption which could be expected to result from increased lactic acid oxidation following infusion of isoprenaline (Fig 1 and 3) In the experiments with 0 05 μg isoprenaline the area below this theoretical curve constituted 97 per cent of the experimentally determined oxygen consumption in those with 0 5 μg it constituted 109 per cent It seems, therefore that increased lactic acid may be largely responsible for the calorigenic effect of isoprenaline in ex

On the other hand had elerator action and it
is highly probable th t partially responsible
for the augmented adrenaline did not
accelerate the heart in doses which
stimulated lactic acid a more
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in the higher doses (0 3—0 5 μg/kg/min) — probably raised the cardiac output by increasing the stroke volume thus stimulating in these doses the cardiac activity

We observed in experiments on humans (Lundholm and Svedmyr to be published) that isoprenaline stimulated the oxygen consumption to a greater degree than did adrenaline although the respective effects on lactic acid production were nearly equal Since moreover there emerged a distinct correlation between the stimulatory effect of isoprenaline on oxygen consumption and that on heart rate the calorigenic effect of isoprenaline — at least in man — may be partially ascribed to its cardioaccelerator action

The catecholamines also increase the plasma level of free fatty acids and this effect has been supposed to be of significance for the calorigenic effects of catecholamines in cold adapted (Hannon Evonuk and Larson 1963) and newborn mammals (Moore 1963) The eventually significance of an increase fat oxidation for the calorigenic effects of catecholamines in normal adult rabbit will be discussed in a later paper

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Uterine Motility of the Estrogenized Rabbit

I Isotonic and Isometric Recording in vivo Influence of Anesthesia and Temperature

By

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Abstract

Seteklev J *Uterine motility of the estrogenized rabbit I Isotonic and isometric recording in vivo Influence of anesthesia and temperature* Acta physiol scand 1964 62 68—78 — A technique for isotonic and isometric recording of uterine contractions *in vivo* is described. No significant difference was found in the spontaneous contractions recorded simultaneously from the two horns by the two methods On the other hand differences were found in the responses to gradually increasing stimuli such as increasing concentrations of noradrenaline or of increasing strength of stimulation of the hypogastric nerve The isotonic response rapidly reached a maximum whereas the isometric response increased more gradually Since the isotonic response can be maximal in spite of submaximal activation, the isometric response is a more reliable quantitative measure which is in agreement with previous findings with *in vitro* recording However for qualitative evaluation of responses near threshold stimulation isotonic recording at low loads is preferable. Anesthetics administered i.v in small doses to lightly anesthetized animals slightly enhanced uterine contractions whereas deep anesthesia and local application of the anesthetics depressed the activity Uterine motility is extremely sensitive to decrease in body temperature

In studies on the myometrial response *in vivo* to distension and to stimulation of the peripheral and central nervous system (Seteklev 1964a, b c and d) some methodological problems were met with Firstly since the blood and nervous supply to the uterus is intact alterations in the general condition of the animal during the experiment may influence the response In the present investigation two such factors which were of particular importance for the subsequent studies were dealt with *viz* the type and degree of anesthesia and the body temperature

Secondly different features of the myometrial response may be brought out by different recording techniques *i e* by either isotonic or isometric methods From the results of *in vitro* experiments Csapo (1954) stressed that the isotonic recording method

gives a less exact quantitative measure of the response than does the isometric method It was of importance to develop techniques for isometric and isotonic recording *in vivo* preferably from the whole uterus and to make a comparison between the two methods

The smooth muscle of the uterus is arranged in an outer longitudinal and an inner circular layer and the forces of the myometrium are directed towards the content of the lumen

Schofield (1954) has devised a method for isometric recording of the rabbit uterus *in vivo* which however is influenced mainly by the longitudinal myometrial muscles Using the techniques described below the resultant force exerted by the various groups of cells on the intra uterine content are measured After filling the uterine horns with saline or Tyrode solution isometric recording was obtained by connecting a closed system to a pressure transducer In the isotonic system the uterine content was expressed during the contraction into a reservoir against constant pressure

The uterine response to nervous stimulation shows considerable species differences (Reynolds 1949 Chapt 12) The rabbit was chosen as the experimental animal because the response in this species is best known from previous investigations and the rabbit uterus gives a similar response to many drugs as does the human uterus The uterus duplex of rabbits also allows a comparison between the isotonic and the isometric contractions in the same uterus because these can be recorded separately and simultaneously from each of the two horns (twin uterus preparation) In this way changes in the animal s condition are eliminated when the responses are compared The uterus was excited either by i v injection of noradrenaline or by hypogastric nerve stimulation

Unlike other muscular tissue the motor response of the uterus is dependent on the cyclic variations of the ovarian hormones The progesterone-dominated uterus exhibits no spontaneous contractions and is unresponsive to stimulation (Reynolds 1949 Chapt 7) However uniform pattern of rhythmic activity can be obtained by estrogenic treatment of ovariectomized rabbits (Cross 1958) Therefore spayed and estrogenized rabbits were used

The present communication is concerned with (i) a description of the technique employed in this and subsequent studies (ii) a comparison between the isometric and the isotonic recording techniques and (iii) the influence of anesthesia and body temperature on the rhythmic contractions

Material and methods

Animals Twenty two adult rabbits (2 5—3 7 kg) were used After the ovariectomy performed under penthobarbital anesthesia (Nembutal Abbott 30 mg/kg b w) the animals were treated with diethylstilbestrol (0 1 per cent 0 5 mg i m) every second day for at least one week before the experiment

Anesthesia The actual experiments were carried out under chloralose urethane anesthesia 40 mg chloralose in 1 per cent solution and 250 mg urethane (25 per cent) per kg b w one half given i v and the rest i m Ether was supplied during the operative procedure when necessary The surroundings of the abdominal incision was infiltrated with 0 5 per cent lidocain (Xylocain Astra) *Spinal anesthesia* was obtained by injection of 1— 1 5 ml of 2 per cent Xylocain intrathecally Immediate flaccidity of the tail and the limb ensued further injection extended the level of the spinal anesthesia Respiratory movement usually ceased after a dose of 2 ml

Chloralose was kindly supplied by E Merck Darmstadt

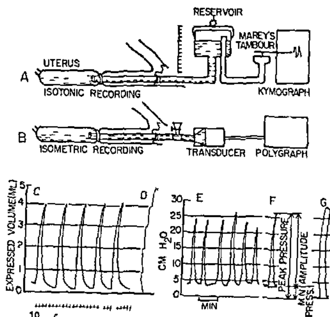


Fig 1 Arrangement for isotonic (*A*) and isometric (*B*) recordings of uterine contractions The isotonic record (*C*) is calibrated (*D*) in ml fluid expressed by the uterus the intra uterine pressure developed by isometric contractions is measured in cm of water (*E*) *F* Definitions of the amplitude the peak pressure and the minimum pressure The mean values were calculated from recordings within a certain time interval (5 or 10 min if possible) *G* Increase of intra uterine pressure after injection of 0 01 ml of fluid into the tube connected with the transducer when the other end of the tube was closed (See text)

Infusion of l noradrenaline Freshly made solutions of l noradrenaline hydrochloride (Nor exadrin conc O N Astra) in Tyrode solution (1 µg/ml) were kept at 0 C Doses from 0 01 µg to 1 µg/kg b w were given through the left saphenous vein

Operative procedures and recording techniques

Ovariectomy was performed through an incision of about 1 cm in the lateral abdominal wall

Adrenalectomy was carried out in two steps The right adrenal gland was excited through an incision of about 5 cm just beneath the costal margin Subsequently the right ovary was removed through the same incision The left adrenal gland was removed during the actual experiment after the incision had been extended to the processus xiphoides

Tracheotomy was routinely performed to secure free respiration

Blood pressure was recorded from the carotid artery by a closed fluid system to a phosphobronze bellows connected to an ink pen writing on kymograph paper The system was provided with a T tube connected to a mercury manometer for calibration In later experiments the blood pressure was recorded by a Statham transducer (Model P 23 AC) connected to a Grass Polygraph (Model 5C)

Isotonic recording of uterine contractions The uterus was exposed through a 5 cm incision in the midline of the abdomen Through a small cut in the cervical end of the posterior vaginal wall a latex tube was inserted through the cervix to about the upper third of the uterine horn (Fig 1) The tube was kept in place by a ligature around the cervix and in some instances by another ligature around the uterine horn The opening of the tube had a diameter of 2 mm Care was taken not to disturb the vascular and nervous supplies to the uterus The latex tube was carried through the vagina and connected to a reservoir again connected to a Marey's

tambour or a Grass pressure transducer (Model PT5A) The abdominal muscles were transected to prevent them from changing the intra uterine pressure. Finally the abdominal opening was closed The latex tube and the uterus were filled with 0 9 per cent saline or Tyrode solution (NaCl 160 mM KCl 2.5 mM NaHCO, 11 9 mM NaH PO 0 32 mM $CaCl_2$ 1 8 mM $MgCl_2$ 0 5 mM and glucose 20 mM)

During an isotonic contraction the luminal content is displaced aga nst a resistance th intra uter ne pressure which is given as the vertical difference between the fluid level in the reservoir and the uterus The amplitude of the record depends on the amount of flu d expelled Calibration was made after occlusion of the latex tube by injection of fluid in steps of 1 ml (Fig 1 D) Because of the considerable larger diameter of the reservoir the flu d expelled from the uterus d d not elevate the flu d level of the reservoir s gnificantly In this way a constant intra uterine pressure was ma ntained during the recording

Isometric recording of uterine contractions By this method the myometrium exerts its power against an incompress ble flu d and the pressure developed was recorded by a Statham transducer (Model P23AC) (Fig 1 B) Fluid could be injected into the system through a three way valve. The pressure was calibrated in cm H_2O by connecting the latex tube and the transducer to the water reservoir used in the isoton c recording (Fig 1 E)

The d stensibility of the latex tube was determined af er closing the uterine end of the tube and by the inject on of 0 01 ml of fluid This amount of fluid being only 1/500 of the operational content of the system caused a greater rise of the pressure than that developed by the spontaneous contractions (Fig 1 G) Therefore the recordings can be cons dered to be virtually un nfluenced by distension of the tube

The amplitude of the contractions was meas ired from the rising point to its peak and expressed as the mean valu of the contractions occurring with n a period of 5 to 10 min The frequency of contractions was expressed as the num ber of contract ons per minute When possible these were also counted with n a 5 or 10 min period

The term minimum press ire has been introduced for descriptive purposes and defined as the lowest pressure between successive contract ons (F g 1 F) Peak pressure is taken as the total press ire reached by the contract ons (minimum pressure plus contraction amplitude = peak pressure) The mean min mum pressure as well as the mean peak pressure were calculated in the same way as the mean ampl tude

Results

In preliminary experiments it was conf rmed that the uterus of progesterone treated rabbits and of rabbits with developed corpora lutea were inactive

By inspection of the exposed uterus of estrogenized rabbits after laparatomy its horns and tubes were usually seen to exhibit slow pendular movements In addition contraction rings were formed in the tubes the uterus and vagina and these usually passed downwards At other times the contraction rings were more stationary When such rings were formed the uterine surface turned pale The uterus reacted by vigorous contractions to mechanical stimuli sometimes temporarily preventing the latex tube from being inserted and the tube had to be anchored to the cervix by ligatures

The frequency and amplitude of these spontaneous contractions varied from animal to animal When the uterus was exposed by laparatomy the spontaneous activity usually subsided but it was soon restored when the abdomen was closed Ord narily the spontaneous rhythmic contractions as recorded isotonically or isometrically were of a fairly regular shape and frequency throughout the experiment As seen from Fig 1 C and F there was no significant difference in the contraction pattern between the isotonic and the isometric recordings The rhythm c contract ons had the same appearance whether 0 9 per cent saline or the Tyrode solution were used Further they were not sensitive to small ariations of the content of the intra uterine fluid Increase in the NaCl-concentration up to 6 per cent did not influence the rh thmic contractions

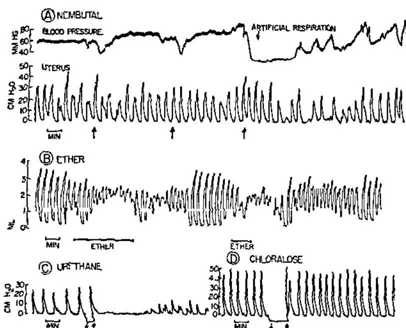


Fig 2 *A* Influence on blood pressure and rhythmic uterine activity of repetitive injections of 5 mg/kg of Nembutal (at arrows) After the third injection arrest of respiration (Exp 131) *B* Influence of ether inhalation on the uterine activity *C D* The uterine rhythmic activity when the intra uterine fluid was withdrawn (↓) and replaced (↑) by an equal volume of 12 5 per cent urethane or 0 5 per cent chloralose (Exp 127)

whereas their frequency progressively increased by elevation of the potassium content from 2 5 mM to 20 40 and 80 mM

In long uteri a more smooth and regular recording could be obtained by a ligature around the cornu The fluid filled part was then limited to the upper part of the uterus The ligature also prevented distension of the cervical part of the uterus which sometimes occurred and caused disturbance of the vascular and nervous supplies

Influence of anesthesia on the rhythmic activity

Additional doses of chloralose and urethane were administered i v in five and three animals respectively In animals under light anesthesia small doses of chloralose and urethane resulted in an increased amplitude of the rhythmic contractions and a rise in blood pressure However with the animal under the ordinary surgical level of anesthesia additional administration of chloralose or urethane (10—40 mg/kg and 250—500 mg/kg respectively) slightly depressed the rhythmic contractions

To avoid the general effect of the anesthetics and to obtain a direct local effect on the myometrium in four animals chloralose and urethane (0 5 and 12 5 per cent in Tyrode solution respectively) were used as the intra uterine fluid instead of the Tyrode solution From Fig 2 D it is seen that chloralose had no significant effect on the rhythmic contractions while urethane caused a marked depression (Fig 2 C)

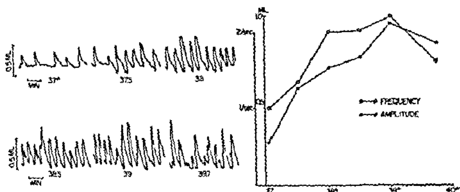


Fig 3 Effects of alteration in body temperature on frequency and amplitude of the rhythmic contractions (Exp 111)

In four animals Nembutal in doses of 6 mg/kg body weight was given in addition to the basic chloralose urethane anesthesia For example in Fig 2 A the first two injections resulted in a transient blood pressure fall but had no significant influence on the uterine contractions Additional injections of Nembutal caused respiratory arrest and a sustained drop in blood pressure demanding artificial respiration During the period of low blood pressure the rhythmic activity decreased but the contraction pattern was restored when the blood pressure rose to normal levels

Intra uterine application of Nembutal (30 mg in 10 ml Tyrode) was found similarly to depress the rhythmic uterine activity without any effect on the blood pressure or respiration

Ether was administered to seven animals in addition to the basic anesthesia The inhalation of ether resulted in an increased minimum pressure increased frequency and smaller amplitude of the rhythmic contractions (Fig 2 B) The activity was resumed 3 to 4 min after the cessation of inhalation In some animals the ether inhalation was followed by a drop in blood pressure associated with a fall in minimum pressure and by decreased amplitude and frequency of the rhythmic uterine contractions

The uterine response to ether administration was not abolished by section of the hypogastric nerves by spinal anesthesia or by adrenalectomy

Spinal anesthesia Instillation of 1 ml 2 per cent Xylocain intrathecally did not reduce the rhythmic uterine contractions but resulted sometimes in a response similar to that obtained by hypogastric nerve stimulation as described below In other cases only a transitional increase in the rhythmic activity ensued The response of the uterus to noradrenaline seemed unaltered Spinal anesthesia was in most cases associated with a moderate decrease in blood pressure

Influence of change in body temperature

The normal body temperature of the rabbit has been given as 3 C Csapo 19 up to 39 6 C Spector 1956 p 437) This discrepancy is perhaps due to the fact that the body temperature in this species falls rapidly during anesthesia unless prevented by high environmental temperature In six rabbits in the present series the rectal tem

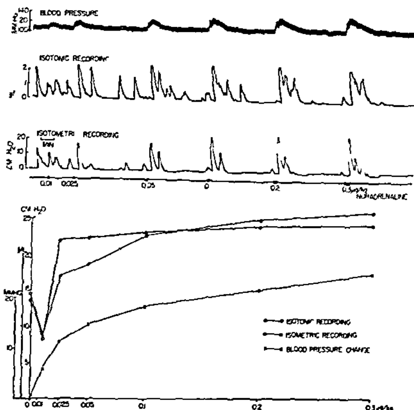


Fig 4 Influence of increasing doses of noradrenaline i v on uterus and blood pressure Simultaneous isotonic (right horn) and isometric (left horn) recordings Below graphic illustration of the same experiment (Exp 107)

perature was measured to be about 39 C (38 7 to 39 3 C) before anesthesia In the choralose urethane anesthetized animals the temperature often fell to about 37 C Warming the animal to 39 C, led to a gradual increase in frequency and amplitude of the rhythmic uterine activity (Fig 3) At higher temperatures (39 7 C) there was a decrease in frequency and amplitude of the contractions

In three other animals the intraperitoneal temperature was lowered by blowing cold air against the abdomen This procedure resulted in a reduction in amplitude and a marked slowing in frequency of the rhythmic contractions The activity was soon restored when the intra abdominal temperature returned to the previous level

Comparison between isotonic and isometric recording

In these experiments a twin uterus preparation was used with simultaneous recording from both horns by the isotonic and isometric techniques respectively

Response to noradrenaline Increasing doses of noradrenaline were administered i v in three rabbits Typical responses are seen in Fig 4 Noradrenaline was in this case injected in doses of 0 01—0 025—0 05—0 1—0 2 and 0 3 μg/kg body weight The lowest dose 0 01 μg/kg caused a slight elevation in blood pressure but a decrease in amplitude

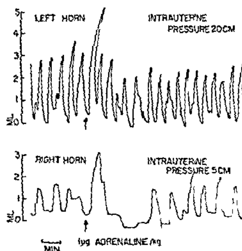


Fig 5 Effect of intra uterine pressure on the isotonic response of a twin uterus preparation to injection of adrenaline 1 µg/kg b w The inhibitory period is best recognized in the left horn where the intra uterine pressure is low (Exp 66)

of the rhythmic contractions in both horns At higher doses the amplitude of the contractions increased As seen from Fig 4 the isotonic response had increased to a fairly constant level even after 0 075 µg/kg The isometric response on the other hand gradually increased with larger doses up to 0 5 µg/kg as did the blood pressure response The additive effect of increasing doses shows that more and more muscular elements are activated Thus the graded isometric response can be regarded as a better quantitative measure of the strength of the myometrial contractions than the isotonic response

It was found that stimuli near threshold gave a more pronounced response when the frequency of the rhythmic uterine activity was low This phenomenon was best studied in the twin uterus preparation in which the background activity could be altered independently in the two horns by variations in the intra uterine pressure (Setekleiv 1964 a) In Fig 5 the response to 1 µg adrenaline/kg body weight is more easily seen in the right horn where the intra uterine pressure was 5 cm of water than in the left horn with 20 cm intra uterine pressure In particular the inhibition was more clearly seen at low pressures

Response to hypogastric nerve stimulation In three animals the peripheral end of the cut hypogastric nerve was stimulated in periods of 5 sec (frequency 50 cps duration of square wave pulses 1 msec) The stimulus strength was increased in steps from 1 to 5 volts (0 16—0 8 mA) The uterine response was recorded simultaneously from both horns one with the isotonic and the other with the isometric recording technique In Fig 6 the intensity was increased from 1 9 to 3 75 volts (0 3—0 6 mA) The intra uterine pressure in the isotonic system was 10 cm of water the volume in the isometric system 2 ml When comparing the two curves the isotonic response is seen to rise rapidly to a maximal value The increase in amplitude of the isometric response is slower and the maximal value is reached later The results are similar to those obtained following increasing doses of noradrenaline Again they indicate that the isometric recording technique allows a better quantitative estimate of the degree of activation of the myometrium

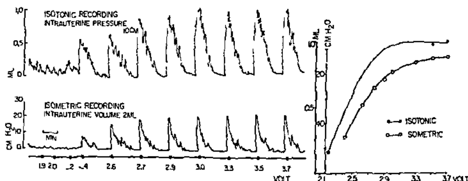


Fig 6 Isotonic and isometric response to increasing strengths of hypogastric nerve stimulation (Exp 109)

Discussion

(1) Comparison between the isometric and isotonic recording techniques

The difficulties in obtaining a reliable recording of uterine contractions have been emphasized In the present experiments the isometric recording has been shown to give a better quantitative measure of the myometrial contractions *in vivo* than does the isotonic technique This is in accordance with observations from *in vitro* experiments (Csapo 1954) *The difference between the isotonic and the isometric response* is greater when just supraliminal stimuli are used The reason is that maximal isotonic shortening may develop although the myometrium is submaximally activated The difference is also dependent on the load in the present experiments represented by the intra uterine pressure and is reduced until the optimum load is reached *i e* the load when the optimum work is performed (Csapo 1954) Since it is difficult to record contractions *in vivo* with small loads this difference is usually small under these circumstances

The maximal response obtained with isotonic recording in spite of submaximal activation can in addition to giving a false picture of the amplitude of the contractions also give an incorrect minimum pressure It was observed often that the contractions in the isotonic recordings lasted longer and the response had a more smooth or tonic appearance than the isometric response

In the method of isometric recording *in vivo* developed by Schofield (1954) a loop of the uterus is fixed in a plexiglass chamber and the shortening of the loop largely produced by the longitudinal muscles is transmitted by a thread to an isometric tension recorder *Similar characteristics of the response to direct electrical stimulation* of the rabbit uterus were found by this method (Schofield 1954 1955) as were described previously from *in vitro* experiments (Csapo and Corner 1952 Csapo and Godall 1954) although the response *in vivo* were less marked and less regular (Schofield 1954) The same method was used by Fuchs and Fuchs (1960) who were not able to reveal any difference between the isotonic and the isometric recording upon oxytocin stimulation

The duration of the isotonic response was *in the present experiments found to be* direct related to the amount of adrenaline or noradrenaline injected and this effect was largely paralleled to the amplitude of the isometric contraction The same result was obtained on stimulating the hypogastric nerve at different frequencies (Setekleiv 1964 b) However the duration of the response is also dependent on the rhythmic activity of the uterus Vigorous rhythmic activity can mask the inhibitory period As the rhythmic activity increases during distension (Setekleiv 1964 a) the uterine response to stimuli near threshold is recognized easier at low intra uterine pressure (Fig 5) Thus for qualitative evaluation of the response to weak stimuli the isotonic recording technique with low intra uterine pressure is preferable

(2) *The spontaneous rhythmic contractions and the effects of anesthesia and temperature change*

With regard to the influence of anesthetics on uterine motility *in vivo* Ferguson (1941) observed no effects of ether chloralose or urethane on uterine contractions in rabbit Only if deep anesthesia was maintained for a quarter of an hour or more did a slow decline in the rhythmic activity develop This decline was interpreted as being due to a decreased secretion of oxytocin In experiments with estrogenized rabbits anesthetized with Nembutal Cross (1958) found that the spontaneous rhythmic contractions were independent of both neurohypophyseal and extrinsic nervous influence but was depressed by increasing depth of anesthesia

In the present experiments it was found that administration of ether reduces the amplitude of the rhythmic contractions but increases the minimum pressure and the frequency of the contractions Since neither adrenalectomy nor section of the hypogastric nerves nor spinal anesthesia could abolish the response a direct peripheral action is likely The effect resembles the influence of acetylcholine on the rhythmic contractions Acetylcholine has a depolarizing effect on uterine smooth muscle (Marshall 1959) It is not known how ether influences the membrane potential of the myometrial cells but it has a depolarizing effect on nervous tissue (Lorente de Nó 1957 p 73) If ether depolarizes the uterine muscle cells this could explain the observed facilitatory effect on the rhythmic contractions

Chloralose urethane and Nembutal were found to enhance slightly the rhythmic contractions when given i v during light anesthesia This increase in the rhythmic activity may indicate that the uterus *in situ* is exposed to some inhibitory influences When applied locally these anesthetics all had a depressive effect only

The rhythmic uterine activity was shown to be sensitive to a decrease in body temperature A fall in rectal temperature of only 2—3 C from the normal value (39 C) was followed by a marked decrease in the frequency and amplitude of the rhythmic contractions This was also observed by Fuchs and Fuchs (1959) and may explain the rapid waning of the uterine activity after laparatomy In addition it was shown in the present study that a rise in the body temperature above the normal (39 C) diminished the uterine motility The extremely narrow temperature optimum makes it essential to control the body temperature in studies of uterine motility

In *in vitro* experiments Csapo (1954) found that the isometric tension obtained by direct electrical stimulation reached a maximum at 40 C At lower temperatures the response decreased and was practically zero at 10 C This influence of the temperature on the uterine contractions may be due to alterations in the membrane potential which increases slightly when the body temperature is lowered (Kuriyama 1961)

The author is deeply indepted to Professor Birger Kaada to my colleagues and the technical staff at the Anatomical Institute University of Oslo for good working facilities and helpful criticism in this and subsequent studies Dr G Burnstock University of Melbourne is gratefully acknowledged for valuable discussion and advice in preparation of the manuscript

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From the Neurophysiological Laboratory Anatomical Institute University of Oslo Norway

Uterine Motility of the Estrogenized Rabbit

II Response to Distension

By

JOHANNES SETEKLEIV

Received 29 January 1964

Abstract

Setekleiv J *Uterine motility of the estrogenized rabbit II Response to distension* Acta physiol scand 1964 62 79—93 — The myometrial response to rapid distension consists of a quick contraction the early stretch response followed by an increase in the rhythmic activity The latency and amplitude of the early contraction has been studied during increasing degrees of distension and during repetitive distensions The frequency of the rhythmic contractions increases with increasing distension the amplitude and the external work performed by each contraction at first increases and later decreases Data obtained from isometric and isotonic recordings indicate that the pacemaker activity is not directly related either to the intra uterine volume or to the intra uterine pressure but rather to the tension in the myometrial cells The independency with which the two horns respond to distension the lack of effect of local and spinal anesthesia and of autonomic blocking agents indicates that the active response to distension is of myogenic origin The intra uterine pressure is not maintained during constant distension but decreases at first rapidly later more slowly

In a previous communication (Setekleiv 1964 a) a technique for isotonic and isometric recording of contractions in the whole rabbit uterus *in vivo* was described The methods employ distension of the uterine horns with saline or Tyrode solution It is known that such distension may influence the spontaneous rhythmic contractions (Reynolds 1949 Chapt 11) but there is no detailed analysis of the response to uterine distension or stretch available Furthermore most previous experiments have been carried out with *in vitro* preparations using the isotonic recording technique which has been shown to be less quantitative than the isometric method (Setekleiv 1964 a) It was therefore necessary to study the effect of distension on the rabbit uterus *in vivo* using the isometric recording technique

It has been shown in other types of smooth muscle that quick stretch may elicit a contraction as well as influencing rhythmic activity This contraction was first observed by Hill (1926) in isolated smooth muscle from *Holoturia nigra* and in *in vivo* experi

ments by Bozler (1947) who worked with the cat ureter Burnstock and Prosser (1960) have recently observed the same type of quick response in isolated strips of smooth muscle from various visceral organs Uterine tissue has not been studied with respect to this early mechanical response With the isometric recording technique used (Seteklelv 1964 a) the reaction could be elicited by quick distension of the whole uterus *in vivo* In the present communication the effect of increasing degrees of distension and of repetitive distension on this response is reported

Further the mechanisms underlying the uterine response to distension have been studied The response may be due to a hormonal or a nervous mechanism or it may be of myogenic origin In a twin uterus preparation a hormonal agent would influence both horns in the same way Therefore if each of the two horns responds independently to distension a hormonal mechanism can be excluded The reflex nature (spinal or peripheral reflex arc) can be proven or disproven by studying the effect of spinal anesthesia of cholinergic or adrenergic blocking agents and of local anesthesia on the response to distension

Material and methods

The present report is based on experiments on forty four rabbits (weight 2 5—4 kg) ovariectomized at least one week before the recording and treated during this interval with 0 5 ml dietylstilbestrol (0 1 per cent) given i m every second day

Operative procedures and *the techniques for isometric and isotonic recording* of uterine contractions were the same as previously described (Seteklelv 1964 a) Particular care was taken to prevent deviations from the normal body temperature (39 C)

Quick distension of the uterus was achieved by rapid injection of Tyrode solution through a three way valve introduced into the latex tube connecting the uterus with the transducer (Seteklelv 1964 a) The injection rate was held as constant as possible and was about 10 ml per sec The definitions and the calculations of the amplitude the minimum pressure and the frequency of the rhythmic contractions have been described previously (Seteklelv 1964 a)

Anesthesia A mixture of chloralose and urethane was used (40 mg chloralos (1 per cent) and 500 mg urethane (25 per cent) per kg b v) If necessary ether or penthobarbital sodium (Nembutal Abbott) was given in addition during the operative procedure Spinal anesthesia was obtained by injection of 1—1 5 ml lidocaine (Xylocain Astra) 2 per cent intrathecally

Stimulation of the hypogastric nerve was performed by implanted electrodes using square wave pulses of 1 msec duration supramaximal intensity and a frequency of 30 cps The technique has been described elsewhere (Seteklelv 1964 b)

The drugs administered Atropine sulphate (0 1 per cent) served as cholinergic blocking agent and for adrenergic blocking Dihydroergotamin Sandoz and Phentolamine (Regitin Ciba) were used Xylocain (1 or 2 per cent) was applied intrauterinely in order to obtain a local anesthesia

Results

Isometric recording of the uterine contractions is a more reliable quantitative measure of the degree of activation than is the isotonic recording method (Seteklelv 1964 a) When not otherwise stated the isometric technique was used in the present study since it was also found to be more suitable in studies of the myometrial response to quick distension

The myometrial response (as measured by the intra uterine pressure) to quick distension is a complex one For descriptive purposes some terms have been introduced in this study for the various components These appear from the diagram in Fig 1 During the injection the tube to the transducer was either open or closed In studies of the early response of the myometrium to distension it was necessary to keep the tube open and

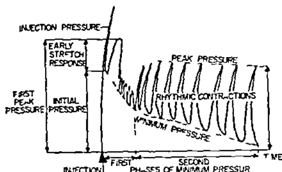


Fig 1 Definitions of the various components of the uterine response to rapid distension Schematic drawing

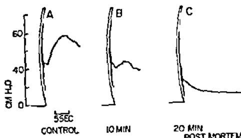


Fig 2 The response to rapid distension (6 ml) The injection pressure is followed by a contraction the early stretch response This is diminished 10 min after the animal has been killed (B) and is absent 20 min post mortem (C) (Exp 142)

the *injection pressure* was recorded Otherwise the recording of this artifact could be avoided by shutting off the connection to the transducer and not turning the valve until immediately after the injection (Fig 5) Following the injection the pressure falls to a level which will be referred to as the *initial pressure* Then follows the first contraction the *early stretch response* The total pressure at its peak will be called the *first peak pressure* This is followed by a series of *rhythmic contractions* The *minimum pressure* is defined as the lowest pressure between successive contractions This pressure gradually falls, first steeply and then more slowly During the first phase of the minimum pressure the rhythmic contractions are of small amplitude and high frequency during the second phase they are of higher amplitude and lower frequency The *peak pressure* is defined as the sum of the minimum pressure and the amplitude of the contractions

The early stretch response

The first peak pressure was shown to consist of at least two components viz the passive resistance of the uterine wall producing the initial pressure and a contraction of the myometrium The presence of a myometrial contraction was demonstrated by *comparing the response to rapid distension in the living and the dead animal (Fig 2)* Such post mortem studies were performed in four rabbits at various intervals and up to 18 hours after the animal had been killed by i v injection of air In the experiment illustrated in Fig 2 the response to quick distension was elicited every 5 min after the animal had been killed After 10 min only a small pressure elevation was present and this was completely absent after 20 min The initial pressure was increased after 5 min

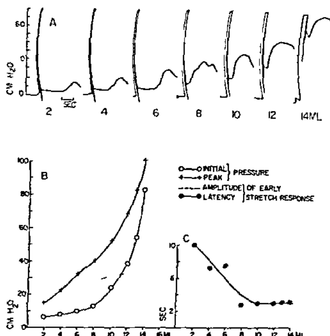


Fig 3 Effect of increasing uterine distension *A* Control showing the changes in amplitude and latency of early stretch response Degree of distension indicated below each record *B* Plot of peak pressure + — + initial pressure 0 — 0 and early stretch response x — x versus degree of distension *C* Plot of latency of early stretch response versus distension *B* and *C* are from the same series of observations as in A (Exp 14?)

later it decreased The increased stiffness of the myometrium may be due to asphytic secretion of adrenaline A maximal contraction of the uterus was always observed when the animal was killed

The early stretch response was also absent in a rabbit spayed one week before recording and treated with 5 mg progesterone (Luteolin© Nyco) each of the two last days

The behaviour of the early stretch response to (a) varying degrees of uterine distension and (b) successive equal distension at varying intervals were studied in eight animals The recordings were obtained at a relatively high paper speed (0 6 mm/sec or more) and with free communication to the pressure transducer as well as to the uterus during the injection

(a *Degree of distension* In the experiment illustrated in Fig 3 the intra uterine volume was increased in steps of 2 ml, from 2 to 14 ml All fluid was withdrawn within 20 sec after each injection and a period of 60 sec elapsed between successive distensions The initial pressure increased with increasing degrees of distension at first slowly and later more steeply Such a relation between the distension and the intra uterine pressure is to be expected if the initial pressure was due mainly to the passive viscous-elastic properties of the uterine wall If the initial pressure and the first peak pressure are plotted against the uterine volume a pressure volume diagram is obtained (Fig 3 B) The amplitude of the early stretch response as measured from the beginning of its rising phase to its peak reached a maximum at a distension of about 10—12 ml and then decreased Fig 3 B) The latency of the early stretch response was measured from the start of injection to the beginning of its rising phase With increasing distensions up to 8 ml there was a gradual decrease in the latency from about 10 to 3 sec (Fig 3 C)

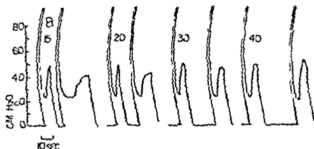
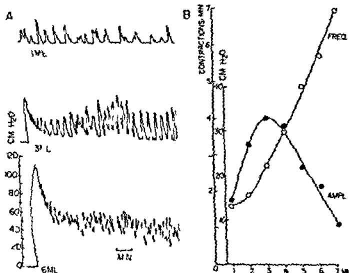


Fig 4 The early stret h response to a second distension applied at varying intervals (1) 20 30 and 40 sec) aft r the first me Volume injected each time was 6 ml The latency is increased at 15 and 20 sec s interval (Exp 1?2)



F g 3 Effects of ncreasing degree of uterine distension on minimum pressure and amplitude and frequenc of th induced rhythmic contract ons 4 Response to rap d inject on of 1 3 and 6 ml Tyrode solution in a series of increas ng injections in steps of 1 ml from 1 to 7 ml B Mean frequency and mean amplitude of the hythmic ontractions during the second phase of m nimum pressure decrease at vari us degrees of distens on The values a e calculated from the experimen s n A in a 10 min period start ng at the beginn ng of the second phase Exp 98)

At greate distension up to 14 ml) the latency remained fa rly constant Similar results as those reported were also obtained in four other animals The shortest latency meas ured was 1 7 sec

(b *Repetitive distensions* The amplitude and latency of the early stretch response were found to depend on the interval betw een successive di tension In the experi

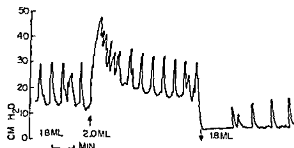


Fig 6 Decrease in minimum pressure and complete inhibition of the rhythmic activity after reduction of the intra uterine fluid (Exp 118)

ment illustrated in Fig 4 the uterus was distended with 6 ml fluid at time intervals varying from 15 to 40 sec between two distensions It can be s en that the latency of the second response increased and the amplitude decreased by shortening the interval between the two successive distensions the initial pressure being relatively constant in all trials except the last one (cf below) At intervals of less than 15 sec between the two stimuli the response to the second stimulus was completely abolished This period of unresponsiveness was found to vary somewhat from animal to animal it was to some extent influenced by the degree of stretch applied being shorter at the greater distensions

After a 40 sec interval there was an increase in amplitude of the second early stretch response Such potentiation was more clearly demonstrated in other experiments However the concomitant fall in initial pressure should be taken into account since it may provide a more favourable condition for the early stretch response

In addition to this potentiation there were also some cyclic variations in the initial pressure as well as in the latency and amplitude of the early stretch response These variations were found to be of the same frequency and in phase with the spontaneous rhythmic contractions and may be due to cyclic changes in the excitability of the myometrium In the interval between expected spontaneous contractions the initial pressure was lower and the latency longer than were the corresponding values at the peak of the expected contractions On the other hand the amplitude of the early stretch response was small when the initial pressure was high and *vice versa*

The rhythmic contractions

In the experiment illustrated in Fig 5 the uterus was distended by a series of rapid injections at constant speed in steps of 1 ml from 1 to 7 ml The findings are representative of those obtained in a series of six animals with the purpose of studying the total uterine response to increasing degrees of distension in isometric recordings At the relatively slow recording speed used in these experiments a separation of the injection pressure from the first peak pressure is difficult The former was therefore eliminated by closing the tube to the transducer during the injection The uterus was allowed to adapt to each volume increase for a period of 10 min the fluid was then withdrawn and the uterus allowed to rest for several min This procedure also served as a control that no fluid had escaped from the organ during the distension

An early rapid and a second slower fall in minimum pressure was clearly seen in most experiments (Fig 5) The transition between these two phases of the minimum pressure was usually fairly distinct The rate of fall in minimum pressure increased at the

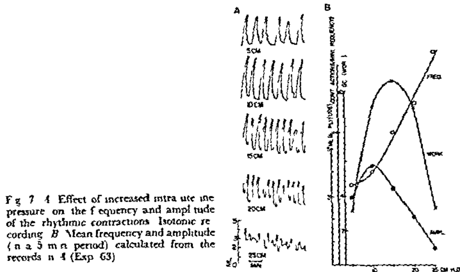


Fig 7 4 Effect of increased intra uterine pressure on the frequency and amplitude of the rhythmic contractions Isotonic recording *B* Mean frequency and amplitude (in a 5 min period) calculated from the records in 4 (Exp 63)

higher degrees of distension In the second phase it appeared to be nearly constant for a given volume However in these experiments the distensions were restricted to 10 min At larger volume this period is too short to obtain a stable level of the minimum pressure The fluid was withdrawn to avoid changes in the preparation which might spoil the comparison of the responses

As mentioned above the induced rhythmic activity which followed the early stretch response showed two distinct patterns in the two phases of the response In the first phase the contractions were of high frequency and small amplitude in the second phase the rhythmic contractions were of lower frequency and higher amplitude (Fig 5) The frequency of the rhythmic contractions was about 10 per min in the first phase and was relatively unaffected by the degree of distension and by the absolute pressure (Fig 5) In general the amplitude decreased with increasing distension In the second phase the mean frequency of the rhythmic contractions increased with increasing distension (Fig 5 B) The amplitude increased up to a certain volume (3 ml in the experiment reproduced in Fig 5) and then decreased

After withdrawal of fluid the intra uterine pressure fell to a level below that recorded before the instillation of this amount (Fig 6) This decrease in intra uterine pressure often resulted in total abolition of the rhythmic contractions For example in the experiment in Fig 6 rhythmic activity was abolished for about 90 sec The contractions reappeared as the minimum pressure increased but the frequency was lower than for the same volume during distension.

The experiments so far described were performed with the isometric recording technique in which the rhythmic contractions were studied following changes in the intra uterine volume By this method it is not possible to obtain a constant rhythmic activity at a given volume The reason may be that the rhythmic activity bears some relation to the continuous decline in minimum pressure However a nearly constant intra uterine pressure can be obtained by the isotonic recording technique It was therefore of interest

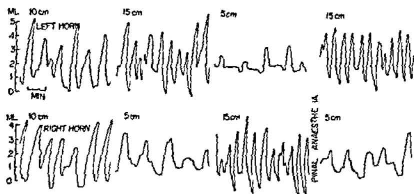


Fig 8 The independence with which the two uterine horns react to distension Isotonic recording in a twin uterus preparation The intra uterine pressure was varied inversely in the two horns Increase in intra uterine pressure in one horn increases frequency and amplitude of the rhythmic contractions in the same horn Spinal anesthesia (right) does not affect the myometrial response (Exp 60)

to compare the behaviour of the rhythmic activity when the distension was performed by an increase in the intra uterine pressure

In ten animals the intra uterine pressure was increased by heightening the reservoir in steps of 5 cm This procedure resulted in similar changes in the frequency and amplitude of the rhythmic contractions (Fig 7) as were obtained with the isometric technique (Fig 5) The frequency increased with developing distension and the amplitude increased to a maximum and then decreased The work performed (amplitude × intra uterine pressure) similarly reached a maximum but at a higher degree of distension

However during a prolonged period with constant intra uterine pressure the frequency and amplitude of the rhythmic contractions were not unaltered Such long term changes were investigated in a twin uterus preparation with isometric recording in one horn and isotonic recording in the other The former was distended with 3 ml Tyrode solution in the latter the intra uterine pressure was adjusted so (10 cm of water) that by inspection through the abdominal incision the horns appeared to be equally distended before the recording started Both horns were then left untouched for a period of 80 min Initially the frequency was the same in both horns In the isotonic record the frequency increased gradually and the amplitude of the contractions decreased By inspection after the experiment this horn was found to have dilated by comparison with the other horn In the latter horn the isometric contractions were nearly absent after 80 min i e the uterus had adapted to the volume

These findings suggest that neither a constant intra uterine volume nor a constant intra uterine pressure can induce rhythmic contractions with a regular frequency and amplitude During the constant volume condition the tension is decreased due to the viscous elements in the muscle during the conditions of a constant intra uterine pressure the tension in the muscle cells is increased due to distension of the uterus (Laplaces law) Thus the tension in the muscle cells may be the common factor which determines the frequency of the pacemaker of the rhythmic contractions

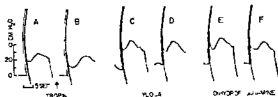


Fig 9 The early stretch response elicited by rapid distension (5 ml) in a uterus filled with 2 ml Tyrode solution before (A) and after (B) i v administration of 1 mg kg atropine before (C) and after (D) the intra uterine Tyrode solution (4 ml) was changed with equal amount of 2 per cent Xylocain and before (E) and after (F) i v injection of 0 6 mg/kg dihydroergotamine In C E and F the distension was performed with 3 ml Tyrode solution Atropine and dihydroergotamine have a slight depressive effect whereas the early stretch response is uninfluenced by Xylocain (Exp 144)

Mechanisms underlying the response to distension

Twin uterus preparation If the uterine response to distension is mediated via a hormonal mechanism it could be expected that in a twin uterus preparation both horns would behave in the same way

By applying different degrees of distension to the two horns they were found to respond independently of each other A representative experiment one of four is shown in Fig 8 Initially the intra uterine pressure in both horns was 10 cm of water and the frequency and the amplitude of the rhythmic contractions were similar on both sides When the intra uterine pressure in the left horn was raised to 15 cm of water the frequency and the amplitude of the rhythmic contractions of this horn increased On the right side the pressure was simultaneously lowered to 5 cm which resulted in a decrease in amplitude and frequency in this horn When the intra uterine pressure was reversed in the two horns opposite alterations in the rhythmic activity occurred These experiments demonstrate that a circulating hormonal agent cannot be the major cause of the response to distension

The latency of the early stretch response may be as low as 1 7 sec The time taken for a circulating hormone to activate the uterus is about 10 sec (Setekleiv 1964 c) which makes it unlikely that the mechanism is due to a circulating hormone

Spinal anesthesia Distension of the uterus is known to produce discharge in the afferent nerves from the uterus (Bower 1959) and this could be the afferent limb in a spinal reflex However spinal anesthesia did not abolish the uterine response to distension This applies both to the rhythmic activity (Fig 8) as well as the early stretch response Even if the intratheceal injection produced respiratory arrest necessitating artificial ventilation the early stretch response persisted A spinal reflex arc for the response can therefore be excluded

Effects of autonomic blocking agents The myometrial response to distension may represent a peripheral reflex mediated through ganglion cells in the peripheral autonomic plexus or within the uterine wall If the response to distension is caused by such a reflex it would be prevented by autonomic blocking agents or by local anesthesia

Atropine sulphate (0 1 per cent) was administered to four animals through the saphenous vein in doses of 1 mg In two cases this dose had no influence neither on the blood pressure or on the spontaneous rhythmic uterine activity in the two other animals there was a transient fall in blood pressure and a concomitant reduction of the amplitude of the rhythmic activity Atropine did not block the early stretch response In three animals, rapid distension of increasing degree was performed before and after admin

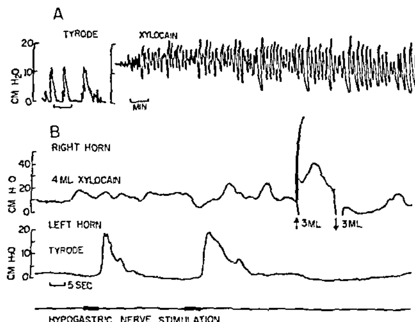


Fig 10 *A* The rhythmic uterine activity before and after intra uterine application of 1 per cent Xylocain The uterine content in both cases 3 5 ml (Exp 116) *B* The early stretch response can still be elicited after the response to hypogastric nerve stimulation (6 V (0 96 mA) 30 cps 1 msec 5 sec) has been blocked by intra uterine application of Xylocain The effectiveness of hypogastric nerve stimulation is secured by simultaneous recording from the other horn (Exp 144)

istration of 1 mg atropine/kg The initial pressure was only slightly influenced by this agent compared with the controls (Fig 9 A and B) The latency of the early stretch response was prolonged after atropine whereas its amplitude was reduced The unresponsive period following one distension was increased from 13 to 18 sec following injection of atropine in the case just referred to (Fig 9) However the early stretch response was elicited also after larger doses of atropine (5 mg/kg)

Regitin and *dihydroergotamine* were used as blocking agents for adrenergic transmitters In four animals Regitin in doses of 10 mg was injected into the saphenous vein in four other animals dihydroergotamine in doses of 1—2 mg was administered in the same way Both drugs lowered the blood pressure but while dihydroergotamine depressed the spontaneous rhythmic activity Regitin increased the frequency and the amplitude of the rhythmic contractions

The early stretch response was not blocked by these agents although a slight depressive influence on the various components was obtained expressed by a slight reduction of the initial pressure and of the amplitude of the early stretch response and a prolongation of the latency (Fig 9 E and F)

Effect of local anesthesia With the purpose of blocking the nerves in the myometrium Xylocain (1—2 per cent) was applied intra uterinely in five animals Replacement of the Tyrode solution with Xylocain enhanced the rhythmic activity considerably (Fig

10 A) The minimum pressure increased after 1—2 min followed by a rhythmic activity of high frequency

Xylocain blocked the response to hypogastric nerve stimulation (Fig 10 B) but the early stretch response persisted In experiments with repetitive distension (Fig 9 C and D) the initial pressure as well as the latency and the amplitude of the early stretch response showed small variations from the controls In one rabbit the early stretch response was elicited when the uterus had been exposed to Xylocain for 1 1/2 hour

Conclusion No evidence is obtained from the results described that the uterine response to distension involves nervous mechanisms Results are more in favour of a myogenic origin The slight depressive effects of atropine Regitin and dihydroergotamine are due most likely to their direct action on the myometrium

Discussion

The present study demonstrates that the estrogenized rabbit myometrium like most other smooth muscle tissues responds to rapid stretch with an active contraction (the early stretch response)

The size of the early stretch response was found to depend on the degree of the distension This variation is presumably due both to changes in the strength of the stimulus and to the mechanical properties of the muscle The decrease in latency indicates that the stimulus is not maximal at small degrees of distension At greater distension the latency is fairly constant However the myometrium is not maximally activated except at high degrees of distension This is supported by an observation which will be dealt with in a subsequent paper (Setekleiv 1964 b) If the myometrium is activated by hypogastric nerve stimulation which is timed so as to coincide with an early stretch response of minimal latency additional pressure is obtained Only at high degree of distension it is not possible by hypogastric nerve stimulation to activate the uterus additionally In addition to these variations in stimulus strength the amplitude of the early stretch response is also influenced by the change in length of the muscle fibres *i e* the length tension relationship

The response to quick stretch may serve a purpose during partus Movements of the foetus with stretch of the uterine wall are believed to act as local stimuli for initiation of contractions in labor (Cross 1959)

The present study has confirmed that the spontaneous rhythmic contractions are influenced by distension Newton (1930) observed that the frequency of oxytocin induced contractions in excised strips of guinea pig uteri was enhanced by increasing the stretch of the preparation The amplitude of the rhythmic contractions in the present investigation was shown at first to increase to a maximum and later to decrease by gradual increase of distension A similar feature of the amplitude was obtained by Kurzel 1928 with isolated whole guinea pig uteri In *in vivo* experiments with cats Simone (1935) found that the amplitude of the rhythmic contractions diminished on increasing the distension

It was found in the present study that there are considerable differences in the rhythmic activity between the first and the second phase of the minimum pressure decrease following a distension of the uterus The explanation is not clear It may be that in this first period some muscle cells are stretched more than others and thus act as a

pacemaker with a high frequency Another explanation is that the high frequency contractions of the first period are due to a long lasting wave of depolarization

Whatever the underlying mechanism may be it seems to be a dynamic and a static response to stretch in the myometrium The dynamic response includes the quick response and the rhythmic activity in the first phase the static response the change in rhythmic activity in the second phase of the minimum pressure decrease

In the second phase a direct relationship was found between the distension and the frequency of the rhythmic contractions However a constant frequency was not maintained either at constant intra uterine volume or at constant intra uterine pressure In either case the tension of the muscle cells is altered and this indicates that the frequency of the rhythmic contractions depends on the tension in the muscle cells Similarly the depolarization and the spike activity induced by stretch of isolated taenia coli was not found to be a function of the length but of the tension produced (Bulbring 1955)

The purpose of the rhythmic contractions during distension is to expell the contene of the lumen These contractions increase with increasing distension *i e* when more work is required However when the uterus is overdistended as may occur, *e g* in polyhydramnion the working capacity of the uterus is reduced

The present observations of the independence of the responses in the two uterine horns (the early stretch response as well as the increase in rhythmic contractions) the independence of spinal reflex arcs and the lack of effect of autonomic blocking agents and of local anesthesia strongly suggest a myogenic origin of the uterine response to distension From recordings of the electrical activity in experiments with ureter Bozler (1947) postulated a myogenic origin of response to distension in this organ too

As regards the cellular mechanisms involved in the response no particular evidence can be obtained by the present methods but certain inferences can be drawn from *in vitro* studies of changes in membrane potentials of smooth muscle cells during stretch Thus Kuriyama (1961) has shown that myometrial cells of post partum mice or rats *are depolarized by stretch In cells of taenia coli it has been found that the depolarization* increased with increasing degrees of extension (Bulbring 1955 Bulbring and Kuriyama 1963) Furthermore the spike frequency is related to the membrane potential level (Bulbring 1955) as is the frequency and magnitude of the rhythmic contraction (Jung 1959) Therefore it appears likely that the early stretch response is initiated by depolarization of the membrane potential of the myometrial cells Burnstock and Prosser (1961) suggested such a mechanism for the response to quick stretch in smooth muscle from pig oesophagus guinea pig and rabbit taenia coli cat intestine rat and guinea pig vas deferens rabbit bladder and dog retractor penis

The myometrial response to quick distension was found to depend also on the state of excitability of the myometrium when the distension was applied The response showed regular variations corresponding to the expected rhythmic contractions. By intracellular recording from the myometrium the membrane potential has been found to exhibit cyclic variations the rhythmic contractions being induced on the top of the depolarization waves (Marshall 1959) The variations in the early stretch response are therefore most likely due to cyclic changes of the membrane potential When the muscle cells are partly depolarized a weaker stimulus (distension) is required to induce the response

A hormonal origin of the uterine response to distension was postulated by Ferguson (1941) He observed that dilatation of one of the uterine horns in post partum rabbits

caused an augmentation of the motility in the other horn This effect was abolished after removal of the pituitary gland and he interpreted the influence on the uterus to be due to discharge of oxytocin In the present study it was found that in the estrogenized rabbit the rhythmic contractions responded to distension independently in the two horns This strongly suggests that a circulating hormonal agent cannot be responsible for the response

The active response to distension of smooth muscle organs does not appear to have been studied systematically by previous investigators in *in vivo* experiments The changes in intraluminar pressure of the urinary bladder at varying degrees of distension have been recorded by Denny Brown and Robertson (1933) and Remington and Alexander (1955) However these authors do not describe the so-called early stretch response of the present study Furthermore they do not make any attempt to distinguish between the pressure changes due to active muscular contraction and those due to the passive mechanical properties of the bladder wall Therefore their results are not easily compared to those of the present investigation They did find however that after a sudden maintained distension the intraluminar pressure of the bladder declined progressively as if the bladder had adapted to the new volume This is similar to what has been described in the present paper as the progressive two-phased decline of the minimum pressure

A number of factors may be responsible for this decline (1) The gradual reduction in active contraction of the myometrium in the late phase of the early stretch response (2) Internal rearrangement or sliding of the myometrial cells whereby their length is reduced and the tension decreased accordingly (3) The viscous-elastic properties of each myometrial cell These will cause a reduction in tension after a sudden maintained stretch The time course of this decline in tension will be determined by the relative values of the viscosity and the relevant stiffness (*cf* Winton 1930) To determine the relative contribution of each of these factors to the time course of decay of the minimum pressure has not been attempted in the present study for various reasons the most important being the difficulty in assaying the passive mechanical properties of the myometrium

In experimental studies on the myometrium it is of importance to take this fact into account and not equate length with tension

The ability to adapt to the intra luminar content may play an important role in uterine physiology During pregnancy the myometrium is inactivated by progesterone action The uterus can then be distended without interference of the active myometrial contraction and without increase in the intra uterine pressure which would give unfavourable conditions for the foetus

Local anesthetics have been found to augment the rhythmic activity of the ureter (Sleator and Bucher 1955) and of the uterus (Clegg 1963) whereas they have a spasmolytic effect in the gastrointestinal tract (Chron, Olson and Nicheles 1944) The action of local anesthetics is apparently not similar in all kind of smooth muscular tissue In nervous tissue where the effect of Xylocain is better known, it does not alter the potential difference across the cell membrane but the membrane is stabilized and thereby made inexcitable (for references see Rud 1961)

In order to elucidate how the response to distension may be influenced by drugs preliminary experiments have been performed with distensions whilst under the influence of the nervous transmitters, acetylcholine and adrenaline noradrenaline The effect of these drugs on smooth muscle is well known from previous studies Acetyl-

choline applied intra uterinely increases the rhythmic activity in a manner similar to Xylocain However it also affects the quick stretch response The initial pressure is increased, the latency reduced and the amplitude of the early stretch response is increased at smaller and decreased at higher degrees of distension Adrenaline and noradrenaline have an effect similar to acetylcholine with the exception that the latency was prolonged by higher distension, indicating decreased excitability of the uterus On intracellular recording acetylcholine is found to depolarize myometrial cells (Marshall 1959) *Adrenaline exerts a diphasic action it partly depolarizes the membrane* and partly it affects the metabolism by tending to stabilize the membrane (Bulbring 1960) This diphasic action may explain the reduction of the contractile ability by greater degrees of distension whilst under the influence of adrenaline

If the effect of Xylocain was due solely to depolarization one would have expected from the acetylcholine-experiments that it would also influence the stretch response According to Clegg (1963) local anesthetics may prevent the spontaneous leakage of catecholamines from uterine stores and thereby eliminate inhibitory influence on the rhythmic uterine activity Obviously further experiments at the cellular level are needed in order to settle this problem

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The Effect of Oxygen at High Pressure on Thyroid Function in the Rat

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Abstract

Sjostrand J *The effect of oxygen at high pressure on thyroid function in the rat* Acta physiol scand 1964 62 94—100 — Rats were exposed to oxygen at 6 atm daily for 4—5 days and killed on the sixth day The animals showed a very low incidence of convulsions The thyroid weight was decreased ($p < 0.05$) but the uptake of I^{131} in the thyroid was increased ($p < 0.05$) The increase of I^{131} uptake of the thyroid per 100 g body weight per mg thyroid weight was highly significant ($p < 0.001$) in the OHP exposed rats A group of rats subjected to stress by formalin injections were investigated for comparison. The thyroid I^{131} uptake was significantly higher in rats exposed to oxygen at high pressure (OHP) than in rats treated with formalin injections ($p < 0.01$) There was thus an increase in the total amount of I^{131} 24 hours after injection in the thyroid associated with the OHP and it seems probable that this was a specific reaction different from the well known response to non-specific stress

Changes in different endocrine organs have been reported after exposure to oxygen at increased pressure (OHP) It has been found that OHP exerts a stimulative action on the sympathetic nervous system and the adrenals (Bean 1945 Bean and Johnson 1954 Edstrom and Rockert 1962 Edstrom and Rockert (1962) studied the effect of OHP on a number of endocrine organs and reported an adrenal hypertrophy a decrease in the weight of the thymus and an increase in the weight of the thyroid These authors concluded that the observed changes in the nervous and the endocrine systems were characteristic for a non-specific stress reaction However the reported weight increase of the thyroid might have been an expression of a specific effect of oxygen at high pressure

It is of considerable practical and clinical interest to study the thyroid function during exposure to OHP since it is reported in literature that the thyroid influences the severity of reactions following exposure to hyperoxia (Grossmann and Pernod 1949 Smith *et al* 1960) This study was carried out in order to obtain a more complete picture of the effect of oxygen at high pressure on thyroid function

Experimental

72 young male rats (Sprague Dawley) weighing 160—210 g at the start of the experiment were used. All the rats received standardized rat cubes (diet 212, Anticimex) and fresh water *ad libitum* for more than a month before the beginning of the experiment. The temperature was kept at + 19 ± 1° C. Series I and IV were carried out in early springtime and series II and III in summer time. The exposure to OHP were performed according to Edström and Rockert (1962) with some small modifications. The animals were placed five at a time in an illuminated pressure chamber having a volume of 35 liters and provided with a window for inspection from outside. The chamber was first washed with pure oxygen for a period of 2 min after which the pressure was raised under continuous ventilation over a period of 10 min to a maximum of 6 atm absolute (ATA). This pressure was maintained for 10 min under ventilation and was gradually reduced to atmospheric pressure over a period of 10 min.

The accumulation of CO_2 was moderate in these experiments. Assuming a CO_2 production of 5 ml/min/rat and using a chamber ventilation rate of 5 l/min the maximum CO_2 pressure in the chamber was 8 mm Hg (360 ml CO_2 at atmospheric pressure).

In series I 33 rats were employed, 16 control and 17 experimental animals. The rats were given two daily treatments during five consecutive days. In series II, comprising 14 animals. The rats were given two daily treatments during five consecutive days. In series II comprising 14 animals the exposure to OHP was performed in the same way as in series I. A group of rats in series II were exposed to non-specific stress by giving them subcutaneous injections of formalin according to a technique described by Schnurer (1963).

In series III comprising 15 animals the rats were exposed to OHP and formalin twice daily during four days and killed on the sixth day. The interval between the end of the exposure to OHP and formalin and sacrifice is 24 hours longer in series III than in series I and II.

In series IV (10 animals) the experimental animals were exposed twice daily to OHP over a 5 1/2 day period. These animals were killed five hours after the last exposure.

The animals in series I and II received one microcurie of carrier free NaI^{131} (the Radiochemical Centre, Amersham) intraperitoneally at 9 p.m. on the fifth experimental day. The animals were killed 24 hours after the injection of the isotope by bleeding under ether anesthesia. In series III the rats were injected with the same dose at 9 p.m. on the fourth experimental day and killed 48 hours later. In series IV the isotope was given at 9 p.m. on the sixth experimental day and the animals were killed six hours later.

At sacrifice the blood was collected and heparinized. The activity of I^{131} in unseparated serum and in the protein bound fraction (PBI) were determined. PBI^{131} was obtained by separation with an anion exchange column (Amberlite Resin Ira-400). At autopsy the thyroid, adrenals and thymus were cleanly dissected out and immediately placed in weighing bottles and weighed. The lungs, heart and abdominal viscera were inspected macroscopically. After weighing the thyroids were dissolved in 1 ml 10 % NaOH in a water bath at 100° C. The thyroids were homogenized and reliable geometrical circumstances for counting were obtained. The counting was performed with a well type scintillation detector. A standard solution containing 10 % of the administered dose solved in 1 ml was counted at the same time and the uptake of I was expressed in per cent of the administered dose. The activity in the plasma fractions was counted up to 3 000 impulses and the activity of the thyroid above 10 000 impulses. The background was counted up to 1 000 impulses.

Results

No animal in the experimental series showed any significant alteration in behaviour or in weight increase. The average incidence of convulsions per OHP exposure was surprisingly low, i.e. 0.3 per cent. Edström and Rockert (1962) reported an average incidence of 5-12 per cent using nearly the same experimental design. However these authors used exposure times of up to 12 min at 6 ATA and the animals were exposed for periods of up to nine weeks. The higher convulsion frequency in the reported study might be due to the prolonged exposure time at 6 ATA or an increasing convulsion tendency after the first week of exposure to OHP.

TABLE I Body and organ weights in OHP exposed and formalin treated rats
Mean values ± S.E.M.

	Controls	n	OHP	n	Formalin	n
Series 1 5 days exposure and series 4 5 ½ days exposure						
Body weights (g)						
Final	220	21	213	23		
Organ weights (mg)						
Thyroid abs weight	12.9±0.4	14	11.1±0.4	14		
Thyroid per 100 g	6.0±0.2	14	5.4±0.2	13		
Adrenals abs weight	35.2±1.4	9	44.3±1.8	11		
Adrenals per 100 g	15.4±0.8	9	20.5±1.0	11		
Series 2 5 days exposure						
Body weights (g)						
Initial	195	5	200	5	171	5
Final	223	5	234	5	232	4
Organ weights (mg)						
Thyroid abs weight	12.2±0.3	5	11.6±0.7	5	10.5±0.6	4
Thyroid per 100 g	5.5±0.1	5	5.0±0.3	5	4.5±0.3	4
Adrenals abs. weight	34.7±1.1	5	39.4±1.5	5	41.9±2.2	4
Adrenals per 100 g	15.5±0.4	5	16.8±0.6	5	18.2±1.0	4
Thymus abs weight	504.8±12.1	5	473.5±19.4	5	373.1±21.0*	4
Thymus per 100 g	226.4±5.7	5	202.6±9.6	5	161.6±9.9	4
Series 3 4 days exposure						
Body weights (g)						
Initial			172	5	199	5
Final	218	5	197	5	216	5
Organ weights (mg)						
Thyroid abs weight	12.5±0.[illegible]	5	10.6±0.4	5	12.0±0.6	5
Thyroid per 100 g	5.[illegible]±0.2	5	5.4±0.1	5	5.6±0.3	5
Adrenals abs weight	3[illegible].9±1.7	5	34.1±2.0	5	37.0±1.7	5
Adrenals per 100 g	16.9±0.6	5	17.3±1.0	5	17.1±0.7	5
Thymus abs. weight	540.3±19.2	5	430.0±25.0	5	4[illegible]4.7±23.5	5
Thymus per 100 g	248.0±9.3	5	218.6±13.1	5	215.1±10.0	5

p < 0.05 p < 0.01 p < 0.001 (compared with controls)

No experimental animal showed any macroscopically detectable pathological changes in the lungs, heart and abdominal viscera

The weight recordings of body weights and organ weights are given in Table I

The weight of the thyroid of the OHP exposed rats showed a decrease in series I and IV ($p < 0.05$) and a smaller insignificant decrease in series II and III The rats injected

TABLE II I uptake of the thyroid in per cent of the injected dose n control OHP exposed and formal n treated rats The interval between injection and sacrifice is 24 hours in series 1 2 3 and 6 hours in series 4

Mean values ± S E.M

	Controls	n	OHP	n	Formalin	n
S ries 1	10 4±1 2	16	[1]13 2±3 1	17		
Series 2	14 8±1 7	5	17.2±1 9	5	5 4±1 7	4
Ser es 3	6 ?±0 8	5	6 7±0 5	5	4 6±0 3	5
Series 4	8 6±0 8	5	8 4±0 5	5		

p < 0 05 p < 0 01 (compared with controls)

Analysis of variance for series 1 and 2 gives p < 0 05 (compared with controls)

TABLE III St tist cal difference in I uptake of the thyro d in per cent of the injected dose between the OHP exposed and formalin treated rats in series 2 and 3

Mean values ± S E M.

	OHP	n	Formalin	n	P for real difference
Series 2	17 ?±1 9	5	5 4±1 7	4	< 0 01
Series 3	6 7±0 5	5	4 6±0 3	5	< 0 01

TABLE IV I uptake of the thyro d, in per cent of the injected dose per 100 g body we ght and per 10 mg thyroid weight, in control OHP exposed and formalin rats The interval between injection and sacrif ce is 24 hours in all series

Mean values + S E M

	Controls	n	OHP	n	Formalin	n
Series 1	3 4 − 0 3	16	5 2 + 0 4	17		
Series ?	6 ? + 0 7	5	6 4 + 0 6	5	2 3 − 0 7	4
Series 3	2 3 + 0 2	5	3 ? + 0 3	5	1 8 − 0 2	5
Series 4	3 0 + 0 3	5	4 2 − 0 6	5		

p < 0 01 p < 0 001 (compared with controls)

with formalin exhibited a decrease (p < 0 02) in the weight of the thyroid in series II and an insignificant decrease in series III

The weights of the adrenals were significantly increased (p < 0 001 in the OHP exposed rats in series I and IV In series II the increase was not significant and in series III the weights were unchanged in the OHP group The rats stressed with formalin

TABLE V I^{131} level in per cent of the injected dose per ml plasma in the organic inorganic and unseparated fraction of blood plasma 24 hours after an intraperitoneal injection Mean values ± S E M

I^{131}	Controls	n	OHP	n	Formalin	n
Series 1						
Organic	0 59 ± 0 06	17	0 50 ± 0 06	19		
Inorganic	0 46 ± 0 11	7	0 28 ± 0 03	15		
Unseparated	1 21 ± 0 12	7	0 88 ± 0 09	15		
Series 2						
Organic	0 41 ± 0 05	5	0 41 ± 0 04	5	0 37 ± 0 03	4
Inorganic	0 22 ± 0 05	5	0 21 ± 0 08	5	0 31 ± 0 05	4
Unseparated	0 63 ± 0 04	5	0 66 ± 0 03	5	0 68 ± 0 02	4
Series 3						
Organic	0 22 ± 0 02	5	0 20 ± 0 03	5	0 17 ± 0 02	5
Inorganic	0 13 ± 0 01	5	0 16 ± 0 01	5	0 11 ± 0 01	5
Unseparated	0 35 ± 0 02	5	0 36 ± 0 04	5	0 28 ± 0 02*	5
Series 4						
Organic	0 17 ± 0 01	5	0 12 ± 0 03	5		
Inorganic	3 37 ± 0 29	5	2 74 ± 0 11	5		
Unseparated	3 44 ± 0 29	5	2 86 ± 0 11	5		

* $p < 0.05$ (compared with controls)

showed an increase ($p < 0.05$) in the weight of the adrenals in series II and no change in weight in series III The thymus of the rats exposed to OHP showed a 10 per cent weight decrease in series II and III although statistically insignificant in both series As a result of the formalin injections the weight of the thymus showed a decrease in series II ($p < 0.001$) and in series III ($p < 0.05$)

The I^{131} uptake of the thyroid 24 hours after the injection was increased in the OHP group in series I and II with a mean of 30 Analysis of variance of the I^{131} uptake values in series I and II showed an increase in the OHP group ($p < 0.05$)

In series I the increase in I^{131} uptake of the thyroid per 100 g body weight and per mg thyroid tissue was highly significant ($p < 0.001$)

In series IV the uptake was unchanged The I^{131} uptake in rats stressed with formalin was decreased in series II ($p < 0.01$) and series III ($p < 0.02$) In series II and III the difference in uptake of I^{131} between rats exposed to OHP and to formalin injections was significant ($p < 0.01$) as shown in Table III

The plasma analysis showed no significant changes in the different series

Discussion

In the present work uptake of I[131] of the thyroid and thyroid weights were measured as indices of thyroid activities. The adrenal weights were recorded in order to obtain information on any possible stress action of OHP.

It is known from earlier workers that non specific stress diminishes the uptake and release of I[131] of the thyroid (Paschkis *et al* 1950, Bogoroch *et al* 1951, Brown Grant *et al* 1956, Schnurer 1963). This was confirmed in the present study in the rats injected with formalin. The differences in uptake of I[131] in the OHP and the formalin groups were significant in all series which speaks for a specific effect of OHP.

The adrenal hypertrophy after exposure to OHP reported by several workers (Bean 1951, Bean and Johnson 1954, Edstrom and Rockert 1962) was also found in the present investigation. The analysis of the adrenal response showed that the increase in weight quickly returned to control values in 40 hours after the last exposure to OHP or the last formalin injection. There was no significant difference in the adrenal response in the OHP and the formalin groups. Bean and Johnson (1954) have suggested that OHP *per se* constitutes a specific type of stress on the adrenals.

The weight decrease of the thyroid after OHP may seem to be in discord with the results of Edstrom and Rockert (1962) who found an increase after 7—9 weeks exposure with nearly the same experimental design. Tiisala (1962) on the other hand reported a weight decrease of the thyroid of two old rats but unchanged weights in growing and adult rats after exposure to hyperoxia for 48 hours. The apparently discordant results could possibly be explained if one assumes that OHP has a double action on the thyroid gland and makes further assumption that one of the components, the non specific stress, acts mainly at an early stage. Non specific stress, which is known to induce thyroid involution, might be a potent factor in the early part of the experiments but less prominent later on with the animals adapted, while OHP as such acts as a thyroid stimulator and this effect becomes dominant over the non specific stress effect after a few days. It is also to be expected that the functional iodide test will respond more quickly to changes in the stimulation of the thyroid than the weight of the organ; changes in the weight of the thyroid is a comparatively insensitive index of thyroid function (Yamada 1961). This may explain the increased I[131] uptake of the thyroid in the cases where the organ weight had decreased. The possible dual action of OHP could be compared to the similar action of cold, which also gives a combination of a non-specific stress response and thyroid stimulation with the concomitant unusual situation of an adrenal and thyroid hyperfunction (Brown Grant *et al* 1954, Brown Grant 1956 a). On the main point there is an agreement between the present results and earlier studies of OHP effects wherein there have been found signs of non-specific stress combined with signs of increase in thyroid activity. The data presented here indicate that the thyroid function in some way is changed by OHP in spite of its action as a nonspecific stressing agent. However, the mode of action of OHP on the thyroid is still unknown.

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Short Term Effects of Reserpine on Noradrenaline Levels in Skeletal Muscle[1]

By

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Abstract

Sedvall G *Short term effects of reserpine on noradrenaline levels in skeletal muscle* Acta physiol scand 1964 62 101—108 — In the skeletal muscle from the hindlimb of cat noradrenaline disappeared almost completely within 5 to 10 hours after reserpine (5 mg/kg i) When the sympathetic nerve supply to the muscles was cut at a preganglionic level before reserpine was given about 15 % of the noradrenaline was still present after 5 hours This remaining amount then disappeared more slowly during 24 to 48 hours and its rate of disappearance was not influenced by a second dose of reserpine When the preganglionic section of the sympathetic supply was performed 3 hours after reserpine administration it prevented the rapid disappearance of the remaining noradrenaline only to a small extent It is known from previous investigations that the noradrenaline in skeletal muscle is stored almost exclusively in the adrenergic nerves The effects of preganglionic section of the sympathetic nerve supply are presumably due to an interruption of the normal impulse discharge in these nerves The data from the present investigation seem to indicate that noradrenaline in the adrenergic nerves of skeletal muscle is stored in two different compartments One larger fraction disappears within a relatively short time after reserpine Another smaller fraction disappears more slowly but this fraction is rapidly released during impulse activity

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Reserpine inhibits an uptake mechanism for amines in the storage granules of sympathetic tissues (Bertler Hillarp and Rosengren 1961 Carlsson Hillarp and Waldeck 1962 1963 Kirshner 1962 a b Euler and Lishajko 1963) By the inhibition of dopamine uptake into such granules reserpine blocks the formation of noradrenaline (Kirshner 1962 a b Kirshner Rorie and Kamin 1963) Reserpine in small doses also inhibits the release of noradrenaline from isolated adrenergic nerve granules (Euler and Lishajko 1961 b)

None of these effects explain why preformed catecholamines disappear from the tissues after reserpine as shown by Carlsson and Hillarp (1956) Bertler Carlsson and Rosengren (1956) and Holzbauer and Vogt (1956) Several factors could be involved in this process One seems to be the nerve impulse discharge Denervation thus prevents the disappearance of catecholamines from the adrenal medulla after reserpine (Holzbauer and Vogt 1956 and others) and noradrenaline disappears at a slower rate from

[1] A preliminary report of parts of this study was presented at the XXII th International Congress of Physiological Sciences Leiden 19[illegible]

adrenergic nerves that have been decentralized or acutely transected before reserpine (Hertting, Potter and Axelrod 1962, Weiner, Perkins and Sidman 1962, Benmiloud and Euler 1963). Ganglion blocking agents have similar effects (Karki, Paasonen and Vanhakartano 1959, Mirkin 1961, Hertting, Potter and Axelrod 1962).

The role played by the discharge of nerve impulses in noradrenaline depletion from several types of adrenergic nerves after reserpine seems to be small (Brodie *et al.* 1957, Kirpekar, Cervoni and Furchgott 1962, Weiner, Perkins and Sidman 1962, Benmiloud and Euler 1963). In a recent investigation on cats Rosell and Sedvall (1962) studied the circulatory responses to electrical stimulation of vasoconstrictor nerves after reserpine. The disappearance of the responses after reserpine was to a high degree prevented by decentralization and promoted by stimulation of the nerves. As the transmitter substance of the vasoconstrictor nerves is considered to be noradrenaline (Folkow and Uvnäs 1948), the results suggested that the discharge of nerve impulses plays an important role in noradrenaline depletion from these nerves after reserpine.

Noradrenaline in skeletal muscle is stored almost exclusively in vasoconstrictor nerves (Sedvall 1964, Fuxe and Sedvall 1964). Quantitative estimation of the noradrenaline content in muscle thus gives information on the noradrenaline store in these nerves. The present paper deals with the effect of sympathetic decentralization on the disappearance of noradrenaline from skeletal muscle of the cat after reserpine. The results indirectly show the role of the nerve impulse discharge in noradrenaline depletion from vasoconstrictor nerves after reserpine. Data will be presented indicating that the noradrenaline in vasoconstrictor nerves is stored — from the point of view of the action of reserpine and nerve impulses — in two different compartments.

Methods

The study was performed on cats weighing 1.8—4.2 kg. All animals were killed by i.v. air. Control animals not given reserpine were the same as in the paper by Sedvall (1964).

In a first series of experiments 33 animals were anesthetized with urethane (500—1300 mg/kg i.v.) and the trachea was cannulated. The rectal temperature of the animals was maintained at 36—37° C by means of a heating lamp. One of the lumbar sympathetic chains was reached by the anterior approach and transected at a level between L4 and L6. Transection of the chain was made on alternate sides. This operation gives a preganglionic sympathetic denervation of the gastrocnemius muscle (Sedvall 1964) and will be referred to below as decentralization. Reserpine (Serpasil, Ciba, 5 mg/kg) was then administered i.v. The animals were killed after 3, 5, 10 and 24 hours respectively. The gastrocnemius muscle from each hindlimb was immediately removed for noradrenaline analysis (see below).

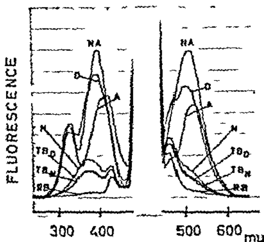
Another [illegible] cats were treated as above but a second dose of reserpine ([illegible] mg/kg i.v.) was administered 5 hours after the first. These animals were killed 10 hours after the first injection of reserpine.

Another 7 cats were operated as above but the sympathetic chain was not transected until 3 hours after the injection of reserpine (5 mg/kg i.v.). These animals were killed 10 hours after reserpine administration.

Fourteen cats were anesthetized with sodium pentobarbital (Nembutal, Abbot 35 mg/kg i.p.) and one of the sympathetic chains was transected as above under aseptic precautions. About 24 hours later, when the animals had recovered from the anesthesia, Reserpine (5 mg/kg i.v.) was injected. These animals were kept at room temperature and killed 1, 3, 10, 24 and 48 hours respectively after the injection of reserpine.

Immediately after death, the gastrocnemius muscle from each hindlimb was rapidly removed for determination of the noradrenaline content. In each cat the muscle with an intact sympathetic nerve supply served as a control. The muscles were homogenized in ice-cold 0.4 N perchloric acid with an Ultra Turrax homogenizer. Homogenization was usually complete within 5 min after death. The muscle extracts were purified and analysed for noradrenaline and adrenaline essentially acc. Bertler, Carlsson and Rosengren (1958) (for details of the method used

Fig 1 Activation and fluorescence spectra (after oxidation and rearrangement in alkali) of synthetic noradrenaline and adrenaline solutions and of extracts from an innervated and from a sympathetically decentralized muscle from a cat 10 hours after injection of reserpine (5 mg/kg i v)
Left Activation spectra Fluorescence wave length 510 mμ
Right Fluorescence spectra Activation wave length 400 mμ
NA Noradrenaline 10 ng/ml
A Adrenaline 10 ng/ml
N Innervated muscle
D Decentralized muscle
TB_N Nonoxidized tissue blank innervated muscle
TB_D Nonoxidized tissue blank decentralized muscle
RB Reagent blank
The muscle extracts were recorded with a higher sensitivity than the standard solutions



Sedvall 1964 In about half of the number of extracts in each experimental series EDTA (1 mg ml) was added to the buffer and to the first 20 ml rinsing water in the ion exchange procedure for extract purification This seemed to improve the reproducibility of the recovery (see below) About half of the number of extracts in each experimental series were analysed according to the improved oxidation procedure devised by Haggendal (1963) Oxidation with the two different methods gave essentially similar results The amounts of noradrenaline have been expressed in terms of free base in nanograms (10 g) per g wet weight of muscle

Model experiments were performed to test the accuracy of the method The mean recovery of 50 ng noradrenaline added to muscle extracts from animals given reserpine (5 mg kg s c) 24 hours before death was in 11 experiments 77 (range 59—89)

Eight duplicate experiments were performed by analysing separately the right and left homologous muscles from 5 urethane anesthetized cats in which both sympathetic chains had been transected at the level of L4—L6 and reserpine (5 mg kg i v) given 5 to 10 hours before death The error of the method for noradrenaline determination calculated on this basis was ± 7 3

To test the identity of the reserpine resistant noradrenaline fraction the activation and fluorescence spectra (after oxidation and rearrangement in alkali) of most muscle extracts were recorded and compared with synthetic solutions of noradrenaline and adrenaline As shown in Fig 1 the spectral characteristics of an extract of a decentralized muscle from a cat given reserpine (5 mg kg i v) 10 hours before death agree fairly well with the synthetic noradrenaline solution.

Results

Sympathetic decentralization had a small but definite effect on the disappearance of noradrenaline from skeletal muscles after reserpine (5 mg kg i v) in the urethane anesthetized cats (Fig 2) In the muscles with a decentralized sympathetic supply there was a rapid disappearance of about 85 per cent of the noradrenaline content within 3 hours The amount of noradrenaline (about 15 per cent of the normal content remaining in these muscles 5 hours after reserpine disappeared however at a considerably slower rate The disappearance curve had thus two principal components Fig 2B The main part decreased to 50 per cent in less than 3 hours while a small fraction decreased to 50 per cent in about 10 hours The disappearance curve for noradrenaline in the muscles

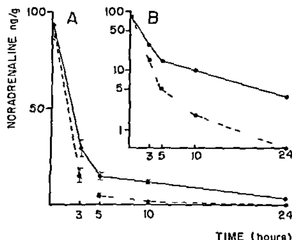


Fig 2 Noradrenaline content (ng/g) of innervated ( ) and sympathetically decentralized (———) skeletal muscles in the urethane anesthetized cat at different intervals after reserpine (5 m /kg i v) In A and B the same results are presented but in B the noradrenaline content is plotted on a logarithmic scale Each point is the mean of determinations from at least six cats Vertical bars represent ± S E

with an intact sympathetic supply also seemed to have two components but the slowly disappearing fraction was considerably smaller After 10 hours there was less than 2 per cent of the noradrenaline left in the innervated muscles The main effect of sympathetic decentralization was thus to protect a small fraction of the noradrenaline content from rapidly disappearing after reserpine The effect of decentralization is illustrated also in Fig 1 This figure gives a comparison between the activation and fluorescence spectra of extracts from a decentralized muscle and from the homologous innervated muscle from a cat given reserpine 10 hours before death It is evident that the decentralized muscle contains much more noradrenaline than the normally innervated muscle

The data presented in Fig 2 were obtained with the gastrocnemius muscle Similar results were obtained with the tibialis anterior and quadriceps femoris muscles of the cat (unpublished data)

Essentially the same results were found in nonanesthetized cats in which unilateral sympathetic decentralization of the muscles had been performed 24 hours before reserpine administration (Table I) There was a tendency to lower values both in sympathetically decentralized and control muscles from these cats after reserpine in comparison with those in the urethane anesthetized animals At 48 hours after reserpine there was practically no noradrenaline left in either the innervated or the decentralized muscles

To study the acute effect of reserpine on the slowly disappearing noradrenaline fraction a second high dose of reserpine (5 mg/kg i v) was given in another series of animals 5 hours after the first injection of reserpine (Table II) Roughly the same amount of noradrenaline was found in the muscles of animals that had received two doses of reserpine as in those treated with a single dose The second dose of reserpine thus had no effect on the slowly disappearing noradrenaline fraction

The results described show that decentralization performed before reserpine administration protected about 15 per cent of the noradrenaline from rapid disappearance In another series of urethane anesthetized animals decentralization was performed 3 hours after reserpine administration At that time there was still about 15 per cent (16 ± 3 3 ng/g mean ± S E. n = 7) of the noradrenaline content left Seven hours later (i e 10 hours after reserpine administration) there was only 4 6 ± 0 8 ng g (n = 7) in the

TABLE I Noradrenaline content (NA) of innervated and sympathetically decentralized skeletal muscle from nonanesthetized cats at different intervals after reserpine (5 mg kg i.v.) Decentralization performed 24 hours before reserpine administration

No of animals	Hours after reserpine	Innervated side NA (ng g)	Decentralized side NA (ng g
1	1	13	30
3	3	11	23
4	10	1 5	10
3	24	0 [illegible]	2 8
3	48	0 6	0 8

TABLE II The effect of sympathetic decentralization on the noradrenaline content (NA) of the skeletal muscle of urethane anesthetized cats after reserpine (Mean ± S E.

No of animals	Time for decentralization	Innervated side NA (ng g)	Decentralized side NA (ng g)	Difference p
10	10 hours before death	1 9 ± 0 5	1[illegible] ± 1 2	< 0 001
7	10 hours before death	3 ± 1 4	13 ± [illegible]	< 0 01
7	7 hours before death	2 0 ± 0 7	4 6 ± 0 8	< 0 01

Reserpine (5 mg kg) was given i.v. 10 hours before death.
Reserpine (3 mg kg was given [illegible] 5 and 10 hours before death.

decentralized muscles Table II This amount was only slightly but significantly higher ($p < 0.01$) than that in the corresponding innervated muscles 2 0 ± 0 7 ng g n = 7 It was significantly lower ($p < 0.01$) than the amount present 3 hours after reserpine Moreover it was significantly lower $p < 0.001$) than the amount 12 ± 1.2 ng g n = 10; 10 hours after reserpine administration when decentralization was performed before reserpine The 15 per cent fraction remaining 3 hours after reserpine was thus only to a small extent protected by decentralization.

Discussion

Experiments made by Hertting Potter and Axelrod (1962 Weiner Perkins and Sjd man (1962) and Benmiloud and Euler (1963) showed that sympathetic decentralization or acute sympathetic denervation slowed down the rate of disappearance of noradrenaline from adrenergic nerves in various tissues after reserpine The present results support these findings.

The main effect of sympathetic decentralization found here was to protect a small fraction of the noradrenaline content in skeletal muscle from rapid disappearance after reserpine The disappearance curve for noradrenaline in the decentralized muscles had two principal components (Fig 2B The main part of the noradrenaline disappeared rapidly being reduced to 30 per cent in less than 3 hours while a small fraction disappeared with a slower rate decreasing by 50 per cent in about 10 hours A second high

dose of reserpine had no effect on the slow disappearance of this fraction indicating that it is relatively resistant to the effect of reserpine In muscles with an intact sympathetic innervation practically all the noradrenaline disapperared rapidly within about 10 hours (Fig 2) A most important process leading to the disappearance of noradrenaline after reserpine seems to be the rapid metabolism of the substance by monoamine oxidase presumably within the amine storing cell (Kopin and Gordon 1962) The rapid disappearance of the main part of the noradrenaline content of the sympathetically decentralized muscles could reflect this process The slow disappearance of the reserpine resistant fraction suggests that it is stored unavailable for monoamineoxidase activity

The effect of decentralization is presumably due to abolition of the continuous impulse discharge in the adrenergic nerves The slowly disappearing noradrenaline fraction can be rapidly depleted by electrical stimulation of the decentralized adrenergic nerves (Sedvall and Thorson 1963) Thus the major part of the noradrenaline disappeared independently of the nerve impulse discharge while the rapid disappearance of the small reserpine resistant fraction was highly dependent upon nerve impulses

It is now generally considered that reserpine blocks an uptake mechanism for amines in catecholamine-storing structures (Hughes and Brodie 1959 Muscholl 1960 Bertler Hillarp and Rosengren 1961 Dengler *et al* 1961 a b Carlsson Hillarp and Waldeck 1962 1963 Kirshner 1962 a b Euler and Lishajko 1963) The slow disappearance of the reserpine resistant fraction could be explained by a small fraction of the uptake mechanism escaping the action of reserpine A certain amount of amine could then be taken up and retained in the store in spite of the action of reserpine A continuous nerve impulse discharge would tend to depress this amount This hypothesis became less probable when it was found that the noradrenaline remaining in the innervated muscles 3 hours after reserpine was only to a small extent protected from disappearance by subsequent decentralization (Table II) This finding showed that after reserpine the noradrenaline content could actually be depressed to a very low level within 10 hours after administration without the simultaneous effect of nerve impulses There is also the possibility that the persistence of the small fraction was due to a change in the effect of reserpine from one of rapid release to one of slower release (Euler and Lishajko 1961 b) The latter explanation to the present findings can not be excluded but seems unlikely in view of the results of Rosell and Sedvall (1962) which are discussed below

The experimental data seem to be best compatible with the view that noradrenaline in skeletal muscle is stored in two different compartments one larger and reserpine sensitive and the other smaller and reserpine resistant but from which noradrenaline can be released by nerve impulses This would explain 1 why the disappearance curve for noradrenaline in the decentralized muscles had a rapid and a slow component 2 why a small fraction was relatively resistant to the effect of reserpine and 3 why the 15 per cent fraction remaining 3 hours after reserpine was only to a small extent protected by decentralization

A recent study of the adrenergic innervation of skeletal muscle with a specific histochemical fluorescence method (Fuxe and Sedvall 1964) indicated that both the reserpine sensitive and the reserpine resistant noradrenaline fractions are stored in the vasoconstrictor nerves Noradrenaline in adrenergic nerves seems to be present both in granules and in the cytoplasmic sap (Euler and Lishajko 1961 a) Hillarp (1960) has presented evidence that catecholamines in adrenal medullary granules are stored in two different pools The results found here could thus reflect the effect of reserpine on such pools in the adrenergic nerves During recent years several investigators have sug

gested the existence of a reserpine resistant noradrenaline pool in adrenergic nerves (Trendelenburg 1961 Kopin and Gordon 1963) In the present study experimental data are presented indicating the occurrence of such a pool in the vasoconstrictor nerves The data further indicate that the reserpine resistant pool can be released by nerve impulses

The present experiments were performed under conditions similar to those of Rosell and Sedvall (1962) The latter study investigated the disappearance after reserpine of the circulatory responses in skeletal muscle to electrical vasoconstrictor nerve stimulation Pronounced effects could be produced by stimulating the vasoconstrictor nerves to sympathetically decentralized muscles as much as 10 hours after the administration of reserpine when only the small reserpine resistant fraction is left This is in accordance with the results of Trendelenburg and Gravenstein (1958) and Gaffney Chidsey and Braunwald (1963) who found that only a small proportion of the normal noradrenaline content was necessary to produce strong peripheral responses by the stimulation of adrenergic nerves Three hours after reserpine administration the normally innervated muscles still contained about 15 per cent of the noradrenaline content Practically no responses to nerve stimulation could be elicited at that time in similarly treated muscles (Rosell and Sedvall 1962) The noradrenaline amount then remaining in the nerves thus seems not to be available for release by nerve impulses This corroborates the suggestion made above that the noradrenaline in vasoconstrictor nerves is localized in separate compartments

A comparison of the results obtained here and the results of Rosell and Sedvall (1962) seems to show that the responses to vasoconstrictor nerve stimulation after reserpine are better correlated to changes in the reserpine resistant noradrenaline fraction than to changes in the noradrenaline store as a whole This indicates that the reserpine resistant fraction plays an important role in the transmission process

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Acta physiol scand 1964 62 109—114

From the Department of Pharmacology University of Goteborg Sweden

The Effect of Adrenaline on the Hexosephosphate Content of Vascular Smooth Muscle

By

ANDRAS BEVIZ and ELLA MOHME LUNDHOLM

Received 6 June 1964

Abstract

Beviz A and E Mohme Lundholm *The effect of adrenaline on the hexosephosphate content of vascular smooth muscle* Acta physiol scand 1964 62 109—114 — In experiments on isolated vascular smooth muscle adrenaline at a concentration of 10 caused a 2 to 3-fold increase in the content of glucose 6-phosphate fructose-6-phosphate fructose 1 6-diphosphate and dihydroxyacetone phosphate during isometric contraction 7 min after addition of the drug This action of adrenaline was manifest both under aerobic and under anaerobic conditions and in the presence as well as in the absence of glucose Fructose 1-6-diphosphate showed a relatively greater increase than fructose-6-phosphate — Since in previous experiments on vascular muscle no glycogenolytic effect of adrenaline had been detectable the possibility that hexosephosphates was formed from some other carbohydrate metabolite is discussed

It has earlier been demonstrated that adrenaline stimulates the lactic acid production of smooth muscle probably via a primary effect upon the muscle's carbohydrate metabolism (Mohme Lundholm 1953 Lundholm and Mohme Lundholm 1962 a 1963 a) The mechanism of this stimulation however is yet to be elucidated In skeletal muscle and in liver the stimulatory effect of the catecholamines on carbohydrate metabolism has been attributed to increased glycogenolysis resulting from activation of phosphorylase the enzyme which catalyzes the reaction glycogen $\xleftrightarrow{\text{phosphorylase}}$ glucose 1 phosphate. Phosphorylase occurs in an active *a* form and in a *b* form which is inactive in the tissues Phosphorylase *a* can be synthesized from *b* in the presence of ATP and the enzyme phosphorylase *b* kinase This enzyme too can be isolated in an active as well as an inactive form Its inactive form can be activated by Ca ions by changes in pH and by cyclic 3 5-AMP (Krebs Graves and Fischer 1959 Formation of the last named nucleotide is stimulated by adrenaline in the presence of ATP and certain cell fragments (Sutherland and Rall 1960) Phosphorylase *a* is converted to *b* in the tissues by an enzyme phosphorylase phosphatase

The above mentioned enzymes have been demonstrated also in smooth muscle and cyclic 3 5-AMP has been found to activate smooth muscle phosphorylase b kinase (Mohme Lundholm 1963) Thus there is enzymatic evidence that adrenaline has the same point of interference in smooth as in striated muscle In certain types of smooth muscle moreover it activates phosphorylase (Mohme Lundholm 1962) It would seem however that the influence of adrenaline upon phosphorylase is less essential for lactic acid stimulation in smooth muscle than in striped muscle

In striated muscle the glycogenolytic effect of adrenaline was almost twice the lactic acid stimulating effect (Lundholm Mohme Lundholm and Svedmyr 1965) in smooth muscle the former effect equalled the latter only during advanced substrate depletion (Mohme Lundholm 1960) Under normal conditions and during relaxation of the muscle by adrenaline glycogenolysis was considerably less pronounced than lactic acid production (Lundholm and Mohme Lundholm 1957) On contraction of the muscle by adrenaline either the glycogenolytic effect was quite absent or glycogenolysis decreased while lactic acid production increased (Lundholm and Mohme Lundholm 1963 b) Should adrenaline interfere with the carbohydrate metabolism essentially via increased glycogenolysis the intensity of glycogen breakdown would obviously have to equal or exceed that of lactic acid production Adrenaline did not timulate the phosphorylase activity of vascular muscle contracted by adrenaline despite aug mentation of the lactic acid production (Mohme Lundholm 1962)

Should glycogen not be the precursor of the lactic acid formed in smooth muscle after adrenaline that precursor is probably to be sought among the intermediate carbohydrate metabolites It may be asked to what extent these metabolites may give rise to lactic acid formation — Under anaerobic conditions and in the absence of glucose the maximum lactic acid production in various types of smooth muscle exceeded maximum glycogenolysis by 130—150 per cent and approximately 4 μmoles lactic acid per g could be formed from precursors other than glycogen (Lundholm and Mohme Lundholm 1960)

In skeletal muscle (Cori and Cori 1931 Newsholme and Randle 1961 Lundholm *et al* 1964) and in heart muscle (Williamson and Kreisberg 1963) adrenaline augmented the hexosephosphate content — The question was therefore (1) whether adrenaline influenced the content of hexosephosphate in smooth muscle and (2) whether any one of those phosphates was present in such a concentration that it could have been a precursor of the lactic acid not formed from glycogen It seemed *a priori* likely further more that adrenaline acted upon a rate limiting reaction or reactions in the glucose chain In a steady state the concentration of intermediate metabolites would presuma bly be comparatively high before this enzymatic bottleneck but low after it Adrenaline would cause a shift *i e* the concentration would fall prior to the rate limiting reaction and then rise (*cf* Krebs and Kornberg 1957)

In the relevant portion of the glycogenolytic chain the following metabolites (itali cized) were therefore determined

Glycogen → *glucose 1 phosphate* → *glucose 6-phosphate* → *fructose 6-phosphate* → *fructose 1 6-diphosphate* → glyceraldehydephosphate → *dihydroxyacetonephosphate* → lactic acid

The experiments reported below suggest that the method outlined here affords some indication of the point at which adrenaline interferes in the carbohydrate metabolism of smooth muscle

TABLE I Glucose 6 phosphat fructose-6 phosphate fructose 1 6-diphosphate and dihydroxyacetone phosphate contents (in μmoles per 100 g wet we ght) of isolat d bovine mesenteric artery Effect of adrenal ne 10 7 min after administrat on during isometric contraction of the arter al preparat o i P = probabil tv that the effect was due to chance n = number of tests

	Glucose 6 phosphate	Fructose-6 phosphate	Fructose 1 6 diphosphate	Dihydroxyace tonephosphate
Aerob c 0 glucose				
Bas l	1 1±0 4	0 6±0 1	0 9±0 2	0 3±0 08
	(n=11)	(n=11)	(n=8)	(n =8)
adrenal ne	3 0±0 5	1 3±0 1	3 3±0 5	0 8±0 11
ncrease	1 9±0 5	0 7±0 2	2 4±0 4	0 5±0 13
	1 < 0 01	P< 0 01	P< 0 001	P< 0 01
Anaerobic 0 glucose				
basal	0 9±0 2	0 7±0 1	—	—
	(n=5)	(n =5)		
adrenaline	2 1±0 5	1 2±0 1	—	—
increase	1 2±0 4	0 5±0 17	—	—
	P< 0 05	P< 0 05	—	—
Anaerobic 0 5 per cent glucose				
basal	1 0±0 2	0 3±0 06	—	—
	(n=5)	(n =5)	—	—
ad enalin	1 7±0 3	0 8±0 2	—	—
increase	0 7±0 2	0 5±0 15	—	—
	P< 0 05	P< 0 05	—	—

Methods

Bovine mesenteric arteries were used in these experiments From each artery two segments about 5 cm long and weighing 0 4—0 6 g were taken *opened length wise and mounted in* special plastic frames so that the tension was evenly distributed o er the enti e specimen The frames were then set up in isometric containers and the specimens subjected to a continuous load of approx mately 10 g w h ch caused elongation At the end of 30 min the tension was adjusted to 0 g and to one of the two preparat ons was added *adrenaline at a concentration* of 1 10 whereby the tension increased The two muscl specin ens were taken for analysis 7 min later before max mal tension had been reached in the one treated with adrenaline At this juncture the latter specimen s lactic acid productio i was assumed to have risen by about 300 per cent (Lundholm and Mohme-Lundholm 1965) Tyrode solution with or without 0 5 per cent glucose was used for suspension In some experiments the solut on was bubbled with a CO_2-O mixture in others with n trogen

The experiments were term nated bv dipping the preparat ons in Freon 12 at −80 C Those port ons which had been attached to the frames we e cut away the remainder was weighed then homogenized in 8 vol 6 per cent perchloric acid.

Following centrifugation the perchloric ac d extracts were neutralized with potass um carbonate in order to prec p tate the excess of perchloric acid as potass um percl lorate In the smooth muscle experiments where 1 ml extract was taken for analysis quant tative precip tation

The above mentioned enzymes have been demonstrated also in smooth muscle and cyclic 3-5-AMP has been found to activate smooth muscle phosphorylase *b* kinase (Mohme-Lundholm 1963). Thus there is enzymatic evidence that adrenaline has the same point of interference in smooth as in striated muscle. In certain types of smooth muscle moreover it activates phosphorylase (Mohme-Lundholm 1962). It would seem, however, that the influence of adrenaline upon phosphorylase is less essential for lactic acid stimulation in smooth muscle than in striped muscle.

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Should glycogen not be the precursor of the lactic acid formed in smooth muscle after adrenaline, that precursor is probably to be sought among the intermediate carbohydrate metabolites. It may be asked to what extent these metabolites may give rise to lactic acid formation. — Under anaerobic conditions and in the absence of glucose the maximum lactic acid production in various types of smooth muscle exceeded maximum glycogenolysis by 150—250 per cent, and approximately 4 µmoles lactic acid per g could be formed from precursors other than glycogen (Lundholm and Mohme-Lundholm 1960).

In skeletal muscle (Cori and Cori 1931, Newsholme and Randle 1961, Lundholm *et al.* 1964) and in heart muscle (Williamson and Kreisberg 1963) adrenaline augmented the hexosephosphate content. — The question was, therefore, 1) whether adrenaline influenced the content of hexosephosphate in smooth muscle and (2) whether any one of those phosphates was present in such a concentration that it could have been a precursor of the lactic acid not formed from glycogen. It seemed *a priori* likely, furthermore, that adrenaline acted upon a rate-limiting reaction or reactions in the glucose chain. In a steady state the concentration of intermediate metabolites would, presumably, be comparatively high before this enzymatic bottleneck, but low after it. Adrenaline would cause a shift, i. e. the concentration would fall prior to the rate-limiting reaction, and then rise (cf. Krebs and Kornberg 1957).

In the relevant portion of the glycogenolytic chain the following metabolites (italicized) were therefore determined:

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Ae ob c 0 glucose				
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adrenaline	3 0±0 5	1 3±0 1	3 3±0 5	0 8±0 11
increase	1 9±0 5	0 7±0 2	2 4±0 4	0 5±0 13
	P<0 01	P<0 01	P<0 001	P<0 01
Anaerobic 0 glucose				
basal	0 9±0 2 (n=5)	0 7±0 1 (n=5)	—	—
adrenaline	2 1±0 5	1 2±0 1	—	—
ncrease	1.2±0 4	0 5±0 17	—	—
	P<0 05	P<0 05	—	—
Anaerobic 0 5 per cent glucose				
basal	1 0±0 2 (n=5)	0 3±0 06 (n=5)	—	—
adrenaline	1 7±0 3	0 8±0 2	—	—
increase	0 7±0 2	0 5±0 15	—	—
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Methods

Bovine mesenteric arteries were used in these experiments From each arterv two segments about 5 cm long and weighing 0 4—0 6 g we e taken opened lengthwise and mounted in special plastic frames so that the tens on was evenly distributed over the entire specimen The frames were then set up n isometric conta ners and the specimens subjected to a continuous load of app oximately 10 g which caused elongation At the end of 30 min the tens on was adjusted to 0 g and to one of the two p eparations was added ad enaline at a concentration of 1 10 whereby the tens on increased The two muscle spec mens were taken for analysis 7 m n later before maximal tens on h d been reached n the one treated w th adrenal ne At th s juncture the latter specimen s lactic ac d product on was assumed to have risen by about 300 per cent (Lundholm and M hme Lundholm 1965) Tyrod solut on with or w thout 0 5 per cent glucose was used for suspens on In some experiments the solution was bubbled w th a CO O mixture in others with nitrogen

The experiments were terminated by dipping the preparat ons in Freon 12 at —80 C Those portions which had been attached to the frames were cut away the rema nder was we ghed then homogenized in 8 vol 6 per cent perchlori acid

Follow ng centrifugation the perchloric acid extra ts were neutralized with potassium carbonate in ord r to precipitate the excess of perchloric ac d as pot ss um perchlo ate In the smooth muscle experiments where 1 ml extract was taken fo analysis quantitati e prec pitation

was essential for otherwise perchloric acid interfered with the enzymatic reactions For satisfactory precipitation it was necessary following neutralization with potassium carbonate to evacuate the extract *in vacuo* for removal of CO_2 and then to cool it to 0 C

Glucose 1 phosphate was determined *ad modum* Bergmeyer and Klotzsch (1962) glucose 6-phosphate and fructose 6 phosphate *ad modum* Hohorts (1962) fructose 1 6-diphosphate and dihydroxyacetone phosphate *ad modum* Bucher and Hohorts (1962)

When the unknown amount of substance had been determined in the extract the method was tested for quantitative recovery by addition of a known amount of substance to the reaction solution Recovery varied between 95 and 110 per cent of the amount added Any experiments which showed lower recovery figures were excluded on the assumption that the perchlorate had not been quantitatively precipitated

Enzyme solutions and substrates were obtained from Boehringer (Mannheim)

Extinction of the solutions was tested in 40 mm quartz cuvettes with the use of a thermostat controlled recording Beckman DK 2 spectrophotometer

Results

Under aerobic conditions and in the absence of glucose the concentration of glucose 6 phosphate averaged 1 1 ± 0 3 µmole per 100 g wet weight (Table I) The corresponding concentration of fructose 6 phosphate was 0 6 ± 0 09 and of fructose 1 6 phosphate 0 9 ± 0 25 µmole per 100 g Dihydroxyacetone phosphate on the other hand showed a lower mean figure of 0 3 ± 0 08 and in some experiments only a trace of it was detectable (0 1 µmole per 100 g) Although glucose 1 phosphate could be identified its content was too low (approximately 0 1 µmole per 100 g) for conclusive demonstration of variations in concentration by the analytical method as applied here

Under anaerobic conditions the glucose 6 phosphate content was somewhat lower than under aerobic conditions though the difference was not significant Addition of glucose had no appreciable effect on the glucose 6 phosphate content

With the preparations mounted isometrically adrenaline at a concentration of 10 ⁶ increased the content of all intermediates 2 to 3 fold as determined 7 min after addition of the drug The increase occurred under both aerobic and anaerobic condition and in the latter case both in the presence and in the absence of glucose In each instance *the increase was statistically verifiable*

It was of interest to determine whether adrenaline disturbed the metabolite balance by affecting the enzymatic reaction rate

With equilibrium in the reaction glucose 6 phosphate $\xrightarrow{\text{phosphoglucose isomerase}}$ fructose 6 phosphate 68 per cent is present as glucose 6 phosphate and 32 per cent as fructose 6 phosphate (Slein 1955) — For mesenteric artery specimens under *aerobic conditions this percentual ratio was 63 37 before addition* of adrenaline and 67 33 thereafter the difference was not statistically significant The phosphoglucose isomerase reaction is unlikely therefore to be a rate limiting factor in the carbohydrate metabolim of mesenteric artery The reaction fructose 6 phosphate + ATP $\xrightarrow{\text{phosphofructokinase}}$ fructose 1 6 diphospate proceeds only towards the right and is not an equilibrium reaction since fructose 1 6 phosphate is converted via a phosphodiesterase to fructose 6 phosphate — Under aerobic conditions the ratio of fructose 6 phosphate to fructose 1 6 phosphate was 48 52 per cent After addition of adrenaline this ratio changed to 25 75 per cent the difference (22 6 ± 4 9 per cent) was statistically significant ($P < 0\ 01$)

Discussion

The glucose 6-phosphate fructose-6-phosphate fructose 1-6-diphosphate and dihydroxyacetone phosphate contents of bovine mesenteric artery were under aerobic conditions only one tenth to one twentieth of those similar determined in isolated rat diaphragm (Lundholm *et al* 1965) Not more than about 60 per cent of the mesenteric artery tissue consists of smooth muscle Even if we assume that the bulk of the intermediates was localized to the smooth muscle cells the concentrations of those products in vascular muscle were nevertheless much lower than in skeletal muscle The percentual increase in the vascular muscle contents of glucose 6-phosphate, fructose-6-phosphate and fructose 1-6-phosphate was equal to that noted for skeletal muscle (Lundholm *et al* 1965) This finding suggested that in smooth muscle adrenaline stimulated a rate limiting reaction in the carbohydrate metabolism that led to an increased formation of hexosephosphates In experiments on skeletal muscle adrenaline was observed to have glycogenolytic and phosphorylase activating effects In vascular muscle no such effects were noted although the same method was used

The possibility that the hexosephosphates increased because of an inhibitory effect further down the glycolytic chain may be ruled out since under the relevant experimental conditions the lactic acid production was elevated approximately threefold.

Smooth muscle appears to contain in addition to glycogen a precursor of lactic acid with a concentration equivalent to about 4 μmol lactate per gram — In mesenteric artery muscle hexosephosphates showed a concentration of about 0 03 μmole per g and could scarcely have constituted the precursor of the lactate It seems therefore probable that this precursor is to be sought either among certain polysaccharides of low molecular weight that have previously been demonstrated in striated muscle (Beloff Chain *et al* 1955) or among the intermediates of the pentose shunt

The stimulatory effect of adrenaline on the hexosephosphate content of vascular muscle was not secondary to the muscle contraction it persisted after addition of dihydroergotamine which blocked adrenaline s contractile action (Beviz *et al* 1965)

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Acta physiol scand 1964 62 115—118

From the Department of Pharmacology University of Goteborg Sweden

The Time Course of the Disappearance of Noradrenaline and 5-Hydroxytryptamine in the Spinal Cord after Transection

By

NILS-ERIK ANDÉN JAN HÄGGENDAL TOR MAGNUSSON and EVALD ROSENGREN

Received 7 February 1964

Abstract

Andén N E. J Häggendal T Magnusson and E Rosengren *The time course of the disappearance of noradrenaline and 5-hydroxytryptamine in the spinal cord after transection* Acta physiol scand 1964 62 115—118 — The rabbit spinal cord was transected in the upper thoracic region Below the lesion the noradrenaline disappeared almost completely between the third and seventh day after the operation The 5-hydroxytryptamine was depleted between the fifth and seventh day The disappearance of these amines was much slower than that of the noradrenaline in the rabbit iris after excision of the superior cervical ganglion There was no significant increase of the monoamine levels above the transection of the spinal cord

Transection of the spinal cord causes an almost complete disappearance of noradrenaline (NA) and 5 hydroxytryptamine (5-HT) below the lesion (Magnusson and Rosengren 1963 Carlsson, Magnusson and Rosengren 1963) This finding seemed to indicate that there are descending noradrenergic and 5-hydroxytryptaminergic nerve fibers in the spinal cord With the use of a fluorescence method for the histochemical demonstration of catecholamines and 5-HT Carlsson *et al* (1964) have found monoaminergic nerve tracts descending in the spinal cord with the cell bodies in the brain stem and with the terminals mainly in the lateral but also in the anterior and posterior horns There are thus many data supporting the view that both NA and 5-HT serve as transmitters in the central nervous system In the present work we have studied the changes of the levels of these amines above and below a thoracic transection of the rabbit spinal cord and the disappearance of the NA in the rabbit iris after excision of the superior cervical ganglion

TABLE I The levels of noradrenaline and 5 hydroxytryptamine in the rabbit spinal cord at various intervals after transection in the upper thoracic region

Days after transection	Noradrenaline (μg/g)		5 Hydroxytryptamine (μg/g)	
	Above transection	Below transection	Above transection	Below transection
Controls	0.081	0.064	0.28	0.27
	0.097	0.109	0.27	0.30
	0.108	0.079	0.27	0.23
			0.22	0.22
Mean	0.095	0.084	0.26	0.26
1	0.073	0.324	0.23	0.22
	0.059	0.076		
	0.110	0.135		
Mean	0.081	0.178		
2	0.077	0.063	—	—
	0.070	0.083		
Mean	0.074	0.073		
3	0.158	0.090	0.24	0.23
	0.117	0.024	0.30	0.23
Mean	0.138	0.057	0.27	0.23
4	0.118	0.043	—	
	0.108	0.019		
	0.030	0.054		
Mean	0.085	0.039		
5	0.204	0.029	0.37	0.32
	0.084	0.009	0.33	0.20
Mean	0.144	0.019	0.35	0.26
7	0.284	0.009	0.30	0.04
	0.111	0.013	0.38	0.05
			0.23	0.03
			0.28	0.01
			0.31	0.09
Mean	0.198	0.011	0.30	0.04
9	0.183	0.007	0.34	0.07
	0.076	0.006		
Mean	0.130	0.007		

TABLE II The content of noradrenaline in the rabbit irides at various intervals after bilateral excision of the superior cervical ganglion

Hours after ganglionectomy	Controls	4	8	16	24	36	48
Noradrenaline	0.44		0.39	0.45	0.014	0.004	0.009
(µg/2 irides)	0.31	0.38					
	0.49		0.42	0.20	0.008		
Mean	0.41	0.33	0.41	0.33	0.011	0.004	0.009

Experimental

Adult rabbits of both sexes weighing 2—3 kg were used Under ether anesthesia the spinal cord was transected in the upper thoracic region by a pair of scissors after a midline incision Post operatively great care was taken to prevent hypothermia decubitus residual urine and infections At various intervals after the transection the animals were sacrificed The roots and meninges were removed from the cord The NA contents of the cord above and below the lesion were determined according to Haggendal (1963) The 5 HT amounts were determined with the method described by Bertler and Rosengren (1959) *except that the tissue residue was re-extracted* once with the original volume of 0 4 N perchloric acid In the 5 HT experiments the animals were anesthetized with pentobarbital sodium (Nembutal Abbott for veterinary use 20—30 mg/kg i v) and bled to death from a carotid artery at the same time as they received an infusion of oxygenated Ringer s solution at 37 C into an external jugular vein This procedure eliminated the contribution of the 5 HT of the blood platelets to the nervous tissue In the NA experiments the animals were sacrificed by air embolism

In other rabbits the superior cervical ganglion and the adjacent sympathetic chain cranially to the clavicle were removed on both sides in pentobarbital sodium anesthesia After various intervals the animals were sacrificed by an intravenous injection of air The NA content of the two irides was determined according to Haggendal (1963)

Results and Discussion

The results of the spinal cord transection are presented in Table I There was no evident reduction of the NA concentration below the lesion during the first 2 or 3 days There after the NA level was dropping to almost insignificant values nine days after the operation The reduction of the 5 HT level in the spinal cord seemed to be a little different from that of NA There was no major change of the 5-HT concentration below the lesion during the first 5 days In the following 2 days the 5-HT seemed to disappear rather suddenly to almost insignificant values

As is well known many different organs are depleted of NA after removal of the ganglion belonging to their sympathetic nerves (Euler and Purkhold 1951 Goodall 1951) The time course of this NA disappearance in peripheral organs is faster than that in the spinal cord *below a transection* The NA of the rabbit iris began to be depleted at about 16 hours after excision of the superior cervical ganglion and had completely disappeared at 24 hours (Table II) The time course of this disappearance agrees well with that found in the rat (brown fat tissue Wemer Perkins and Sidman 1962 submaxillary

gland Benmiloud and Euler 1963) Also with the fluorescence method for histochemical demonstration of catecholamines approximately the same time course of the NA disappearance in the rat iris has been observed after excision of the superior cervical ganglion (Falck 1962) The sympathetically innervated tissues of the cat seem to demand a somewhat longer time or about 36 hours to be depleted of NA (nictitating membrane Kirpekar Cervoni and Furchgott 1962 hindleg muscles Sedvall 1963) It is interesting that in all these investigations there has been a latency time before a gradual drop of the NA level which also seemed to occur in the spinal cord

With the fluorescence method for histochemical demonstration of monoamines it has been found that the fluorescence of the noradrenergic pathways and cell bodies above a spinal transection become much more intense and that many nerve tracts will be seen which are not visible in non-operated animals (Fuxe personal communication) Some of the present data suggest an increased NA concentration above the lesion but there are also several values in the normal range The problem requires further investigation Maybe the fluorescent pathways even if very intense under the fluorescence microscope do not contribute very much compared with the terminals to the total concentration of monoamines in the spinal cord

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Acta physiol scand 1964 62 119—124

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Adrenergic Mechanisms in the Pupillary Light-Reflex Path

By

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Abstract

Dahlström A K Fuxe N Å Hillarp and T Malmfors *Adrenergic mechanisms in the pupillary light reflex path* Acta physiol scand 1964 62 119—124 — On the basis of the known effects of reserpine on the eye the presence of monoaminergic mechanisms in the light-reflex path of the rat was studied with the use of a fluorescence method for the localization of catecholamines and 5 hydroxytryptamine at the cellular level The nucleus of Edinger Westphal — but not the large-celled nuclei of the oculomotor complex — was found to receive abundant nerve fibres which show a massive accumulation of a primary catecholamine in all probability noradrenaline There is little doubt that the nerves are adrenergic synaptic terminals This adrenergic system is so richly developed that is must be assumed to play an important role in the regulation of the activity of the visceromotor outflow to the eye Except this system and the adrenergic neurons recently discovered (Malmfors 1963) in the retina no other monoaminergic mechanisms have so far been detected with certainty in the light reflex path Abundant fibres of both the adrenergic and 5-hydroxytryptaminergic type terminate in other parasympathetic (e g the dorsal motor nucleus of the vagus) and also sympathetic nuclei (e g the sympathetic lateral column of the spinal cord) 5-hydroxytryptamine and noradrenaline in the central nervous system are thus not confined to action upon the parasympathetic and sympathetic systems respectively — Current views on the central action of reserpine are discussed on the basis of the findings

Bogdanski Sulser and Brodie (1961) have analyzed the effects of reserpine on the eye They found that the extreme miosis induced by this drug is caused partially by the blockade of the adrenergic transmission due to the depletion of the transmitter (Carlsson et al 195) from the sympathetic nerves of the iris but predominantly by a marked increase in parasympathetic activity This increased activity was shown to be due at least partly to a mechanism that operates independent of light stimuli and which was thought to comprize a — possibly direct — stimulation of pupilloconstrictor centres Evidence was obtained however that also another mechanism is involved an increased sensitivity to light enhancement of the light reflex It seems reasonable to assume that the

two latter effects — like the blockade of the adrenergic transmission — are due to the specific action of reserpine on monoaminergic mechanisms (*cf* Carlsson 1964) There is good evidence that monoamines in the central nervous system are formed and stored in monoaminergic neurons (Carlson *et al* 1964 Dahlström and Fuxe 1964) On the basis of the findings made by Bogdanski *et al* (1961) it may thus be predicted that such neurons are connected to both the afferent and efferent part of the light reflex path This has proved to be a good working hypothesis Previously unknown adrenergic mechanisms have been found both in the retina (Malmfors 1963 Haggendal and Malmfors 1963) and in the nucleus of Edinger Westphal

Material and methods

The cellular localization of monoamines in the lower brain stem of about 125 adult male albino rats was examined with the help of a histochemical fluorescence method (Falck *et al* 1962 Falck 1962) as described in detail in another paper (Dahlstrom and Fuxe 1964a) The effects of certain drugs on the monoamines were also studied The drugs were administered intraperitoneally

Reserpine 25 rats 1 or 10 mg/kg 8 and 24 hours

Nialamide 25 rats 500 mg/kg 5 to 6 hours

Reserpine nialamide 10 rats 10 and 500 mg/kg respectively Reserpine was given 18 to 19 hours prior to nialamide and the animals were killed 5 to 6 hours later

m Tyrosine 10 rats 3 doses of 400 mg/kg at intervals of 2 hours The animals were killed 2 hours after the last injection

α Methyl *m* tyrosine 5 rats 400 mg/kg 24 hours

Results

A circumscript and fairly dense accumulation of fluorescent fibres was found to exist in transverse sections through the mesencephalon rostral to the level of the emergence of the oculomotor nerve (Fig 1) The fibres were localized to a nucleus with mainly small nerve cells just below the cerebral aqueduct and dorso-medially to the large-celled oculomotor nuclei There seems little doubt that the group of small cells constitute the nucleus of Edinger Westphal (*cf* Valverde 1962 Zeman and Innes 1963) No or only a few scattered fibres of the same type were present in the surroundings of this cell group or in the oculomotor nuclei which give rise to the motor nerves of the extrinsic eye muscles

The observed fibres were fine and had abundant varicosities (mostly about 0 3 to 0 6 μ thick) which showed an intense green to yellow green fluorescence They were never seen to run for more than a very short distance and often very intimately enclosed the nerve cell bodies In fact they have exactly the same appearance as the synaptic nerve terminals which contain very high concentrations of noradrenaline and belong to adrenergic neurons in other parts of the central nervous system (see Carlsson *et al* 1964 Dahlstrom and Fuxe 1964b) —The fluorescence reaction and the properties of the fluorescent products were examined according to Dahlstrom and Fuxe (1964a) The results gave strong evidence for the view that the fluorescent terminals in the nucleus of Edinger Westphal contain very high concentrations of a primary catecholamine such as noradrenaline or dopamine

A few scattered small nerve cells with a specific green fluorescence of medium intensity in their cell bodies were present in the nucleus of Edinger Westphal No findings

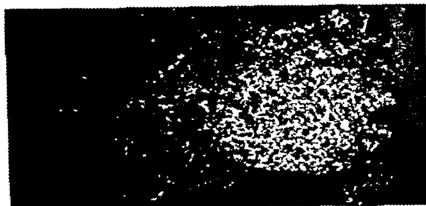


Fig 1 Transverse section of rat lower brain stem at the level of the oculomotor complex Abundant fine varicose nerve terminals with intense fluorescence are accumulated in the nucleus of Edinger Westphal below the cerebral aqueduct seen at the top of the figure 100 x

were made which might suggest that the fluorescent terminals arise from these cells. On the contrary they argue against such a view A group of somewhat larger and more abundant nerve cells also scattered within the nucleus showed a very weak yellow fluorescence or none at all in untreated animals but a strong yellow fluorescence in animals treated with nialamide or reserpine nialamide The destination of their axons is unknown but they were never observed to terminate within the nuclei of the oculomotor complex — The green and yellow fluorescent nerve cells are in all probability adrenergic and 5 hydroxytryptaminergic neurons respectively (Dahlstrom and Fuxe 1964) Several groups of such neurons are present in the lower brain stem

No fluorescent terminals could be observed 8 and 24 hours after administration of reserpine Nialamide treatment did not change their appearance The fluorescence of the terminals did not reappear when nialamide was given to reserpinized animals It disappeared completely after administration of *m* tyrosine Only a few weakly fluorescent fibres persisted after administration of α methyl *m* tyrosine

Other parts of the visual and light reflex paths have also been studied So far no certain findings have been made which might favour the view that monoaminergic mechanisms play an important role in other parts than the retina and the nucleus of Edinger Westphal Since scattered nerve terminals of the adrenergic type have been found in both corpus geniculatum laterale and the anterior colliculus it cannot be excluded however that neurons belonging to the visual system in these regions may be adrenergically influenced

Several other cell groups in the lower brain stem e g the dorsal motor nucleus of the vagus the motor nuclei of the facial and trigeminal nerves and probably also the salivatory nuclei were found to receive more or less abundant nerve terminals of the adrenergic type The vagal and trigeminal nuclei also showed many terminals of the 5-hydroxytryptaminergic type (see Carlsson et al 1964 This will be described in detail in another paper

Discussion

The specificity of the fluorescence reaction the significance of the pharmacological experiments and the identification of synaptic monoaminergic terminals in the central nervous system have been discussed at length in other papers (Carlsson Falck and Hillarp 1962 Carlsson *et al* Falck Dahlstrom and Fuxe 1964b Fuxe 1964) On the basis of the findings in these and in the present study it may be concluded that the fluorescent fibres in the nucleus of Edinger Westphal in all probability are adrenergic synaptic terminals containing very high concentrations of a primary catecholamine almost certainly noradrenaline This group of small nerve cells receives such a wealth of these terminals that the adrenergic system discovered must be assumed to play an important role in the regulation of the activity of the visceromotor outflow to the eye including that of the pupillary constrictor fibres (*cf* Alpern 1962 Lowenstein and Loewenfeld 1962) In contrast to this the large-celled oculomotor nuclei and the other cell groups surrounding the nucleus of Edinger Westphal have no or only few scattered adrenergic terminals It is of interest that also other visceromotor nuclei belonging to both the sympathetic and parasympathetic system receive a monoaminergic innervation of varying abundance Adrenergic and 5 hydroxytryptaminergic neurons in the lower brain stem for instance give two very rich systems of terminals to the thoracolumbar sympathetic column (Carlsson *et al* 1964 Dahlström and Fuxe 1964c) The latter of these seems to be an inhibitory system The dorsal motor nucleus of the vagus also shows abundant synaptic terminals of the adrenergic type The findings that adrenergic neurons converge not only to sympathetic but also to parasympathetic nuclei and that 5 hydroxytryptaminergic neurons give innervation to sympathetic nuclei are of great interest not least with reference to the view that the adrenergic mechanisms are confined to the sympathetic division of both the peripheral and central autonomic nervous system while 5 hydroxytryptaminergic mechanisms are limited to the parasympathetic system in the central nervous system It has recently been shown in this laboratory that sympathetic and certain parasympathetic ganglia as well receive adrenergic synaptic terminals (Hamberger Norberg and Sjöqvist 1963 Norberg 1964) All the direct observations made so far thus contradict this view

It has not yet been possible to determine the origin of the adrenergic system in the nucleus of Edinger Westphal The terminals may however belong to one of the several groups of noradrenaline containing nerve cells found in the lower brain stem (Dahlström and Fuxe 1964a) This possibility is of special interest since the nucleus is subject to inhibitory influences *e g* from the brain stem reticular formation (see Lowenstein and Loewenfeld 1962

As stated in the introductory part it could be predicted that monoaminergic neurons would prove to be connected to both the afferent and efferent part of the light reflex path In agreement with this it was recently discovered that a well defined layer of adrenergic neurons in all probability working with dopamine as transmittor exist in the retina (Malmfors 1963 Häggendal and Malmfors 1963) The neurons are very sensitive to reserpine and there is suggestive evidence that the reserpine induced depletion of their amines results in an enhanced light reflex (Malmfors 1964) No other monoaminergic mechanism has so far been detected with certainty in the visual path The monoaminergic mechanism responsible for the increased pupillary constrictor tone which is induced by reserpine but independent of light stimuli must be localized somewhere in or indirectly connected to the efferent path Since no monoaminergic synaptic terminals

seem to exist in the ciliary ganglion (Malmfors 1964) the extensive adrenergic system in the nucleus of Edinger Westphal is the most likely candidate The prediction is thus fully substantiated

It has been argued especially by Brodie and co-workers (Brodie and Shore 1957 Brodie Spector and Shore 1959) that reserpine due to its blocking of the monoamine storage in the brain produces an increase in free and active amines and that these amines cause the central effects of reserpine On the basis of this view the increased parasympathetic activity in *e g* the pupillary constrictor fibres in reserpinized animals is thought to be due to an excitatory action of free and active 5 hydroxytryptamine exerted on the central parasympathetic system (Bogdanski *et al* 1961 Costa and Pscheidt 1961 Brodie and Costa 1962) Noradrenaline on the other hand is postulated to modulate the central sympathetic system The findings in the present and previous papers do not support these lines of thought however The monoaminergic mechanisms in the light reflex path have so far been found to be adrenergic and not 5 hydroxytryptaminergic It has further been shown — as stated above — that 5-hydroxytryptamine and noradrenaline in the central nervous system are not confined to action upon the parasympathetic and sympathetic systems respectively

The findings in the present paper and the effects of reserpine on the pupillary light reflex seem to be more adequately explained on the basis of the views of Carlsson and co-workers (see Carlsson 1964) as regards the mechanism of reserpine action Since the reserpine induced depletion of noradrenaline from the peripheral sympathetic nerves leads to a loss of the adrenergic transmission (Carlsson *et al* 1957) and the monoamines in the central nervous system are formed and stored in neurons which in all probability are monoaminergic (see Carlsson *et al* 1964 Dahlstrom and Fuxe 1964a) this school thinks it reasonable to conclude that the depletion of the monoamines by reserpine is followed by blockade of transmission also in the central nervous system Consequently the adrenergic mechanisms detected in the light reflex path should cease to function — and not be activated — after administration of reserpine The only assumption now necessary to obtain a logical explanation of all the observations is that the adrenergic neurons in the retina and the adrenergic terminals in the nucleus of Edinger Westphal constitute two inhibitory systems It is well known that this nucleus is subject to inhibitory influences and the existence of an inhibitory mechanism in the retina does not seem unlikely A transmission blockade of these two systems should results both in an enhanced light reflex and in an increase in the light independent pupilloconstrictor activity If this view is correct it is understandable why no 5-hydroxytryptaminergic terminals could be detected in the nervous paths examined since it no longer is necessary to postulate that the parasympathetic system of the eye is activated by free 5 hydroxytryptamine increased through the action of reserpine

Evidence of a more direct nature is obviously needed to provide a more solid foundation for the view discussed above It is important first of all to elucidate the relationship between the pharmacological effects of reserpine and its action on the adrenergic systems detected in the lightreflex path and to establish whether these systems are inhibitory

The motor nucleus of the facial nerve was found to receive many adrenergic nerve terminals This is of interest since reserpine elicites a marked blepharospasm According to Bogdanski *et al* (1961) the blepharospasm is due to an enhanced light reflex It is quite possible however that a blockade of an inhibitor system connected to the efferent part of the reflex path would result in an increased motor outflow in light stimulation.

The adrenergic system also in the nucleus of the facial nerve may thus have an inhibitory function

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Acta physiol scand 1964 62 125—136

From the Institute of Physiology University of Lund Sweden

Identification of a Spinocerebellar Tract Activated from Forelimb Afferents in the Cat

By

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Abstract

Oscarsson O and N Uddenberg *Identification of a spinocerebellar tract activated from forelimb afferents in the cat* Acta physiol scand 1964 62 125—136 — A spinocerebellar tract activated from ipsilateral forelimb nerves has been identified by antidromic activation from the cerebellar cortex. This tract has been denoted the rostral spinocerebellar tract (RSCT) It originates from cell bodies at or slightly above the level of the dorsal roots supplying excitation The RSCT ascends in the middle third of the lateral funiculus where it has a more ventral position than the ventral spinocerebellar tract (VSCT) which it partly overlaps About two thirds of the RSCT enter cerebellum together with the VSCT through the brachium conjunctivum About one third of the RSCT shifts dorsally becomes contiguous to the dorsal spinocerebellar tract (DSCT) and enters cerebellum through the restiform body The RSCT terminates mainly in the anterior lobe but some fibres send branches also to the pyramis and the ipsilateral paramedian lobule In the anterior lobe the RSCT terminates in bilateral zones consisting of a medial strip of the intermediate cortex and a lateral strip of the vermal cortex The termination is more intense ipsilaterally and the fibres reach equally often the hindlimb as the forelimb area — The RSCT receives monosynaptic excitation from high threshold group I muscle afferents and polysynaptic excitation from the flexor reflex afferents The functional organization and the characteristic cerebellar termination suggest that the RSCT is a forelimb equivalent of the VSCT

The anatomy and the functional organization of the dorsal and ventral spinocerebellar tracts (DSCT and VSCT) are known in considerable detail Lundberg and Oscarsson 1960 1962 Grant 1962 b) Both these tracts forward information from the hindlimbs but not from the forelimbs (Grant 1962b Holmqvist Oscarsson and Uddenberg 1963a) Information from the forelimbs would be expected to reach cerebellum through pathways functionally similar to the DSCT and VSCT Recent anatomical and electrophysiological investigations have demonstrated that the cuneocerebellar tract is a forelimb equivalent of the DSCT (Grant 1962a Holmqvist Oscarsson and Rosén 1963b) The present paper describes a spinocerebellar tract which appears to be a forelimb equivalent of the VSCT

Group I muscle afferents in hindlimb nerves activate the DSCT and VSCT but presumably no other ascending tracts (Lundberg and Oscarsson 1960 1962) On the other hand group I afferents in forelimb nerves activate only one ascending spinal tract This tract differs from the DSCT in arising from cell bodies rostral to Clarke s column and in having a relatively ventral position in the cord it differs from the VSCT in being uncrossed (Holmqvist *et al* 1963a)

This investigation is concerned with the course and termination of the group I activated forelimb tract It will be demonstrated that this tract terminates in the cerebellar cortex and hence constitutes a spinocerebellar tract This tract will be denoted the rostral spinocerebellar tract (RSCT) as it is distinct from the DSCT and VSCT and originates from cell bodies in the rostral part of the cord The connections to RSCT neurones made by group I muscle afferents and flexor reflex afferents will be reported in a forthcoming paper Some of the findings have been described in a preliminary report (Oscarsson 1964)

Methods

The experiments were done on cats usually immobilized with gallamine and artificially ventilated The body temperature was kept between 36 and 38 5 C The blood pressure was continually recorded and prevented from falling below 80 to 90 mm Hg

a Antidromic identification The animals were unanaesthetized and decerebrated Fibres belonging to the spinocerebellar tracts were identified by antidromic activation from the cerebellar cortex as described by Lundberg and Oscarsson (1960) The following nerves in the left forelimb were dissected and mounted for stimulation the nerve to the long head of triceps the nerve to biceps the median the deep radial and the superficial radial nerve The left dorsolateral surface of the cord was exposed through a small opening in the caudal part of the lamina of the second cervical vetebra The dura was split and the cord lightly pressed against the roof of the spinal canal by pellets of gel foam inserted beneath the cord outside the dura This prevented respiratory and circulatory movements allowing stable recording from fibres in the lateral funiculus with capillary microelectrodes The afferent volleys were recorded triphasically from the dorsal funiculus at the same segmental level (C3) The medial part of the cerebellum was exposed from the inferior colliculi to the obex and covered with warm mineral oil The exposed area included the vermis the left and sometimes also the right paramedian lobule and medial parts of the hemispheres Square pulses with a duration of 0 2 msec were used for stimulation of the cerebellar cortex A movable silver ball electrode (cathode) was used for scanning the cortex and the indifferent electrode was in the temporal muscle

b Localization in spinal cord and peduncles The animals were under pentobarbitone anaesthesia The following nerves were prepared for stimulation bilaterally the hamstring and gastrocnemius soleus nerves in the hindlimbs as well as the deep radial nerve and the nerve to the long head of triceps in the forelimbs The two muscle nerves in each limb were stimulated in combination in order to produce large discharges in the ascending tracts The afferent volleys were recorded from the dorsal roots in the lumbar region and from the dorsal funiculus at the level of the third cervical segment

The location of ascending tracts in the spinal cord was determined by recording from fascicles of the cord dissected at the C3 level as described by Holmqvist and Oscarsson (1963) The location in the cerebellar peduncles was investigated in decerebrate preparations after sucking away cerebellum The dorsal funiculus was always transected at C3 in order to abolish the discharge in the cuneocerebellar tract (Holmqvist et al 1963b) The brachium conjunctivum was explored by tracking with a needle-electrode mounted in a Horsley Clarke instrument The indifferent electrode was in the temporal muscle The restiform body was prepared for recording as described by Holmqvist et al (1963b) it was dissected free from underlying tissue as far down as to the level of the eighth cranial nerve and mounted on recording electrodes Lesions made in the restiform body were controlled in serial sections prepared with the freezing technique after fixation in 10 per cent formalin

Ipsilateral and *contralateral* refer to the side of the ascending axons

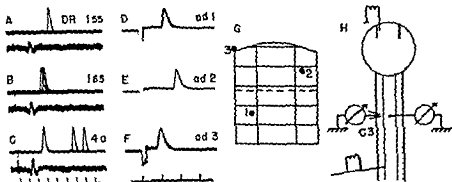


Fig 1 Recording from RSCT unit (upper trace) and from dorsal funiculus (lower trace) at C3 on stimulation of the deep radial nerve (A—C) at strengths indicated in multiples of the nerve threshold D—F show antidromic potentials evoked from the low threshold foci appropriately marked out on the diagram (G) which refers to culmen of the anterior cerebellar lobe (cf Fig 2) Diagram H shows stimulating and recording arrangements The records are formed by superposed traces Time marker msec.

Results

1 Antidromic identification

Ascending fibres were recorded from in the lateral funiculus at the level of the third cervical segment In six experiments 81 fibres were encountered that could be activated antidromically from the cerebellar cortex

1) Thirty-eight fibres were activated from ipsilateral forelimb nerves and classified as RSCT units They occurred in the middle third of the lateral funiculus

2) Thirty five VSCT fibres were identified by their inhibitory and excitatory receptive fields which include both hindlimbs (Oscarsson 1957 Lundberg and Oscarsson 1962) They occurred intermingled with the RSCT fibres and also slightly more dorsally

3) Eight DSCT fibres were identified by their ipsilateral hindlimb fields (Lundberg and Oscarsson 1960) They were found in the dorsomedial part of the lateral funiculus This part was usually avoided as it did not contain any RSCT or VSCT fibres The DSCT fibres terminated in the intermediate zone of the anterior cerebellar lobe as described previously (Lundberg and Oscarsson 1960)

4 Types of RSCT units encountered

Eighteen RSCT units were monosynaptically activated from group I muscle afferents in ipsilateral forelimb nerves and will be denoted A RSCT units These units received almost always weak polysynaptic excitation from the flexor reflex afferents (FRA i e cutaneous afferents and group II and III muscle afferents) For example the unit illustrated in Fig 1 was monosynaptically activated from group I muscle afferents in the deep radial nerve (A B) and received additional excitation from group II afferents in the same nerve (C)

Only polysynaptic excitation from the FRA was observed in the remaining 20 units which will be denoted B-RSCT units Presumably these units received monosynaptic excitation from group I afferents in undissected nerves The termination areas of A RSCT and B-RSCT units are shown on separate diagrams in Fig 2 (B and C The

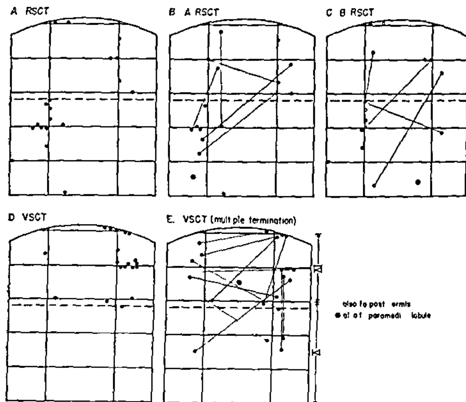


Fig 2 Termination of the left RSCT and VSCT as indicated by the low threshold foci for antidromic activation of individual axons The diagrams refer to culmen of the anterior cerebellar lobe Lars ll s lobules IV and V and the commonly occurring sulci are indicated by horizontal lines The curved line shows the border to the unexposed rostral part of the anterior lobe The interrupted line indicates the border between the hindlimb (rostral) and forelimb area The vermis and intermediate cortices are separated by verti al lines A termination of 38 RSCT units B and C termination of the 18 A RSCT and 20 B RSCT units shown in separate diagrams D termination of 31 VSCT units E termination of the 13 VSCT units with multiple antidromic foci Interconnected points indicate foci belonging to the same unit (B C and E) The few RSCT and VSCT units activated also from the posterior vermis or left paramedian lobul are indicated by open and double contoured circles in B C and E

two groups of RSCT units were similar with respect to location in the cord cerebellar termination and antidromic thresholds and latencies

Twenty units were found in the lateral funiculus that could be monosynaptically activated from group I muscle afferents in ipsilateral forelimb nerves Only two of these units could not be antidromically activated from the exposed part of the cerebellar cortex Presumably these neurones terminated in the unexposed rostral part of the anterior lobe (see below) These observations suggest that all ascending fibres activated from group I muscle afferents in ipsilateral forelimb nerves terminate in the cerebellar cortex The situation is similar to that encountered in connection with the tract units activated from group I muscle afferents in hindlimb nerves which presumably all belong either to DSCT or VSCT (Lundberg and Oscarsson 1960 1962)

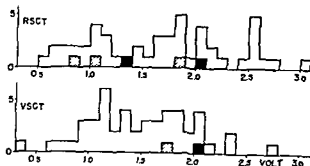


Fig 3 Thresholds (in volts) for evoking antidromic potentials from the low threshold foci of the cerebellar cortex Values relating to antidromic activation from the pyramis and ipsilateral paramedian lobule are indicated by hatched and black squares

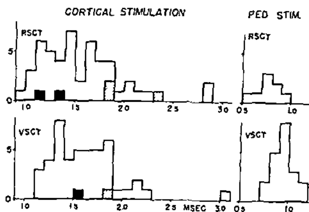
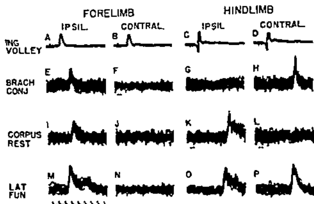


Fig 4 Latencies of antidromic potentials recorded in RSCT (upper) and VSCT units (lower) on stimulation of low threshold foci of the cerebellar cortex (left) and on stimulation of the fibres where they cross the superior cerebellar peduncle (right) The values relate to stimulation at approximately 1 5 times threshold strength Latencies obtained on stimulation of low threshold foci on the pyramis and psilateral paramedian lobule are indicated by hatched and black squares respectively The conduction distance from the superior cerebellar peduncle to the recording site was about 4 5 cm

Furthermore a comparison of the data from the ipsilateral and contralateral side and from the forelimb and hindlimb area indicates that the mode of termination is similar in these various cortical regions The spread of thresholds from 0 3 to 3 0 volts (Fig 3) is to be expected because of the deeply folded cerebellar cortex Though the foci of low threshold antidromic activation do not give more than a rough indication of the termination sites of the individual fibres they can be assumed to give a reasonably good indication of the general extent of the termination areas of the RSCT This is suggested by the good agreement between the termination areas of the DSCT and VSCT as determined by the antidromic method (Lundberg and Oscarsson 1960 1962 this investigation) and by degeneration studies (Grant 1962b)

The latencies of the antidromic spikes varied considerably (Fig 4 left histograms) suggesting a wide range of conduction velocites The spikes evoked in the same unit from different cortical areas usually had different latencies The RSCT unit in Fig 1 was antidromically activated from one ipsilateral and one contralateral point (D E) In this unit the spike evoked from the contralateral termination site had a longer latency than the spike evoked from the ipsilateral site In some other RSCT units the



F g 5 Mass discharges in RSCT DSCT and VSCT recorded from the cerebellar peduncles and the lateral funiculus of the cord Ipsilateral and contralateral muscle nerves in the forel mb (deep radial nerve + nerve to long head of triceps) and h ndl mb (hamstr ng nerve — nerve to triceps surae) ere stimulated at a strength sl ghtly supramaximal for group I afferents The ingo ng volleys f om the two nerves n each limb were synch onized and recorded monophas cal ly from the end of the transected dorsal fun culus at C3 (A B) and triphasically from the dorsal roots at a low lumb r level (C D) The discharges evoked by these volleys are shown in the corresponding lower records E—H record ng with a needle electrode inserted in the dorso-lateral surface layer of the brachium conjunctivum I—L, recording from the restiform body d ssected as descr bed by Holmqvist et al (1963b) M—P subsequent record ng from lateral funiculus dissected at the C3 level Dots ma k stimulus artefacts Time marker msec

reverse v as found Several RSCT units could be antidromically activated from the ipsilateral rostral corner of the exposed part of the anterior lobe (Fig 1 point 3 record F) This is close to the brachium conjunctivum and the fibres were presumably stimulated where they pass across the peduncle before entering cerebellum The VSCT units could more often be activated from the same site which relates to the fact that all the VSCT fibres enter cerebellum through the brachium conjunctivum but only some of the RSCT fibres (see belo) The latencies of the antidromic spikes evoked from the rostral corner were 0 5 to 0 9 msec for the RSCT and 0 7 to 1 1 msec for the VSCT units (Fig 4 right histograms) The time of conduction along the intracerebellar course of the fibres v as calculated by subtracting the above values from those obtained on anti dromic activation from the cortex The conduction times thus obtained were 0 45—1 35 (12 cases mean 0 83) for the RSCT and 0 20—1 30 msec (29 cases mean 0 65) for the VSCT fibres The latency on cortical stimulation usually decreased only little when the strength was increased from slightly supraliminal to 2—3 times threshold suggesting that the slo conduction was not limited to a short terminal part of the fibres

? Locat on of RSCT in the cerebellar peduncles

Volleys in group I muscle afferents evoke large mass discharges in the spinocerebellar tracts from ipsilateral forelimb nerves in the RSCT from contralateral hindlimb nerves in the VSCT and from ips lateral hindl mb nerves in the DSCT These discharges were used for tracing the tracts in the cord and cer bellar peduncles The

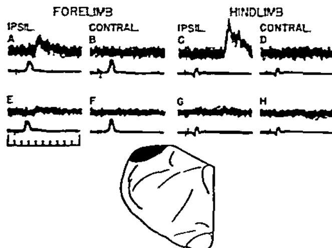


Fig 6 Location of RSCT and DSCT fibres in the restiform body Stimulation of ipsilateral and contralateral muscle nerves in the forelimb (deep radial nerve — nerve to long head of triceps) and hindlimb (hamstring nerve — nerve to triceps surae) at a strength slightly supramaximal for group I afferents. Recording from dissected restiform body (upper traces) and recording of ingoing volleys (lower traces) as described in Fig 5 A—D obtained before and E—H after the lesion (black in diagram made 0.2 cm rostral to the obex. Time marker msec

dorsal funiculus was transected at the C3 level in order to abolish the discharge in the cuneocerebellar tract which enters cerebellum through the restiform body (cf Holmqvist *et al* 1963b) In the experiment of Fig 5 recording was successively made from the brachium conjunctivum (E—H) the restiform body (I—L) and the lateral funiculus at C3 M—P In the peduncles the VSCT discharge was led exclusively from the brachium conjunctivum H L) and the DSCT discharge exclusively from the restiform body G K On the other hand the RSCT discharge was large both in the brachium conjunctivum (E and in the restiform body (I

Similar observations were made in the other experiments of this type The VSCT reached cerebellum through the brachium conjunctivum and the DSCT through the restiform body in accordance with classical concepts At most a few per cent of the fibres might have taken a deviating course through the other peduncle Judging from our five experiments, in which recording was made bilaterally approximately two thirds of the RSCT enter cerebellum through the brachium conjunctivum and one third through the restiform body No potentials attributable to the spinocerebellar tracts were observed in the brachium pontis three experiments

Tracking with a needle electrode in brachium conjunctivum showed that the RSCT and VSCT discharges were optimally recorded from the dorsolateral surface layer of the peduncle and decreased together with displacement of the electrode It is concluded that part of the RSCT accompanies the VSCT through the brachium conjunctivum to the cerebellum.

The location of the RSCT fibres in the restiform body was studied in experiments with recording from that structure dissected rostral to the eighth cranial nerve as described by Holmqvist *et al* 1965b The RSCT and DSCT discharges were recorded

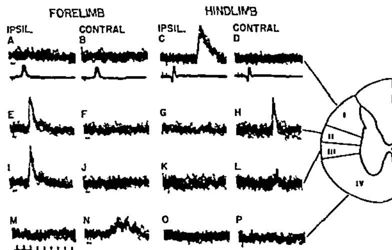


Fig 7 D scharges recorded at the third cervical segment from tracts acti ated by stimulation of *psilateral and contralateral muscle nerves in the forel mb (deep radial nerve* + nerve to long head of tr ceps) and hindlimb (hamstring nerve + nerve to triceps surae) The stimulus strength was sl ghtly supramaximal for group I afferents The records were obtained from the d ssected fascicles I—IV as indicated The lower traces in A—D show the ingo ng volleys re corded as described in Fig 5 The discharge in record N was evoked by impulses n group II afferents Dots mark stimulus artefacts. Time marker msec

before and after lesions made in the restiform body 2—3 millimetres rostral to the obex (five experiments) A large DSCT discharge was recorded in the experiment of Fig 6 (C) together with a moderately large RSCT discharge (A) A lesion in the dorsal part of the restiform body (black in diagram) reduced the resting activity as indicated by a thinner base line (E—H) and almost completely abolished the DSCT and RSCT discharges (E G) It is concluded that the portion of the RSCT that reaches cerebellum through the restiform body and the DSCT are contiguous already at a low bulbar level There was some evidence that the RSCT fibres extended further ventrally than the DSCT fibres (e g Fig 6) but even a superficial lesion in the dorsal part of the restiform body produced a marked reduction of the RSCT as well as of the DSCT discharg

3 Location of RSCT in the sp nal cord

Previous experiments suggested that the RSCT ascends in the middle third of the lateral funiculus separate from the DSCT and partly overlapping the VSCT Holm qvist *et al* 1963a) The observation that the RSCT at the peduncular level is divided into one part contiguous to the VSCT and one part contiguous to the DSCT raised the question whether any trace of this subdivision could be demonstrated in the spinal cord We have therefore repeated the previous experiments with recording f om fascicles dissected at the level of the third cervical segment

In Fig 7 four fascicles (I—IV) were dissected as indicated on the d agram The DSCT discharge was recorded exclusively from the dorsal fascicl I) The VSCT discharge was large in fascicle (II) and ery small in fascicle I whereas the RSCT discharge

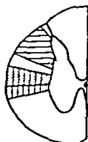


Fig 8 The spinal cord sectors containing DSCT (horizontal hatching) VSCT (stippled) and RSCT (vertical hatching) at the level of the third cervical segment.

was approximately equally large in fascicles (ii) and (iii) No discharge evoked from group I muscle afferents was recorded in the ventral fascicle (iv)

The results from the six experiments made during the present investigation together with those reported previously (Holmqvist *et al* 1963a) demonstrate that the DSCT VSCT and RSCT occupy approximately the sectors shown in Fig 8 The RSCT overlaps the VSCT to a larger extent than assumed before (Holmqvist *et al* 1963a) but there is a small area between the DSCT and RSCT which only contains VSCT fibres It is concluded that part of the RSCT during its further ascent in the cord and medulla oblongata separates from the rest shifts dorsally and joins the DSCT fibres to enter the restiform body

4 Conduction velocity of RSCT fibres

The latency difference of the discharges recorded at the level of the peduncles and approximately 4 0 cm more caudally at the C3 level was used for calculating the conduction velocity of the fastest fibres in the tracts The mean values from five experiments were 95 105 and 82 m sec for RSCT VSCT and DSCT respectively The antidromic potentials evoked in RSCT fibres by stimulation at the brachium conjunctivum had latencies of 0 5—0 9 msec (Fig 4) Without correction for utilization time or electrical spread of stimulus these values would correspond to conduction velocities of 90—50 m/sec The values are approximate because of the short conduction distance but permit the conclusion that the RSCT fibres conduct at velocities comparable to those in the DSCT and VSCT

These observations suggest that the RSCT fibres have large diameters like the fibres in DSCT and VSCT The total number of fibres is presumably of the same order in RSCT and VSCT The mass discharge of these two tracts had usually a similar size when recorded from the dissected lateral funiculus (Fig 5 M—P) Furthermore RSCT and VSCT have a comparable organization with regard to activation from group I afferents (Oscarsson 1964 and were in our experiments activated from nerves of similar size

Discussion

A rostral spinocerebellar tract distinct from the previously known dorsal and ventral spinocerebellar tracts must now be recognized This tract is activated from ipsilateral forelimb nerves and originates from cell bodies at, or slightly above the level of the dorsal roots supplying the excitation (Holmqvist *et al* 1963a) The RSCT ascends in the middle third of the lateral funiculus separated from the DSCT and partly overlapping the VSCT About two thirds of the RSCT enter cerebellum together with the

VSCT through the brachium conjunctivum. The remaining part of the RSCT shifts dorsally, becomes contiguous to the DSCT, and enters cerebellum through the restiform body. The RSCT terminates mainly in the anterior lobe but some fibres send branches also to the pyramis and the ipsilateral paramedian lobule.

Why has a major tract like RSCT, comparable in size with the dorsal and ventral spinocerebellar tracts, remained unrecognized until now? The explanation lies in the difficulty of separating RSCT anatomically from the DSCT and VSCT. In the spinal cord the RSCT ascends intermingled with the VSCT. However, in its rostral course the tract divides into one part that reaches cerebellum together with the VSCT and another part that reaches cerebellum together with the DSCT. Furthermore, the RSCT terminates not only in the somatotopically appropriate forelimb area but also, to an approximately equal extent, in the hindlimb area. This explains why the existence of a spinocerebellar tract related to the forelimb was missed by Grant (1962b) in his thorough investigation of the degeneration in cerebellum that follows lesions in the spinocerebellar tracts.

Besides the DSCT and VSCT some previous authors have described a third tract connecting the spinal cord with the cerebellum (Pellizzi 1895, Beck 1927, Anderson 1943). This tract, the intermediate spinocerebellar tract, is however not identical with the RSCT. The intermediate tract is not related specifically to the rostral part of the cord and has variously been described as reaching cerebellum through the brachium pontis (Pellizzi 1895), the restiform body (Beck 1927) and the brachium conjunctivum (Anderson 1943). Busch (1961) described a group of intermediate spinocerebellar fibres which derive from the cervical enlargement and occupy an area between the DSCT and VSCT at low bulbar levels. Most of these intermediate fibres move into the restiform body at more rostral levels than the DSCT. They are presumably identical with some of the RSCT fibres that enter cerebellum through the restiform body, though it should be recalled that most of these fibres have joined the DSCT already at a level 2 mm rostral to the obex (Fig. 6).

The RSCT can be regarded as a forelimb equivalent of the VSCT as will be elaborated in a forthcoming paper on the functional organization of the tract. Both RSCT and VSCT are monosynaptically activated from high threshold group I afferents, presumably identical with tendon organ afferents (Holmqvist *et al.* 1963a). Individual neurones in both tracts receive convergence of monosynaptic excitation from synergic muscle groups at different joints, suggesting that the tracts forward information concerning stages of movement or position of the whole limb rather than information concerning changes of tension in individual muscles (Oscarsson 1960, 1964, Eccles, Hubbard and Oscarsson 1961). Furthermore, the termination of the RSCT in the cerebellar cortex is similar to that of the VSCT but characteristically different from the termination of the DSCT and the cuneocerebellar tract (Lundberg and Oscarsson 1960, 1962, Grant 1962a, b). However, it should be observed that the RSCT differs from the VSCT not only anatomically but also in some respects functionally. For example, in the RSCT polysynaptic effects from the flexor reflex afferents are weak and excitatory rather than predominantly inhibitory as in the VSCT.

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From the Neurophysiological Laboratory Anatomical Institute University of Oslo Norway

Uterine Motility of the Estrogenized Rabbit

III Response to Hypogastric and Splanchnic Nerve Stimulation

By

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Abstract

Setekleiv J *Uterine motility of the estrogenized rabbit III Response to hypogastric and splanchnic nerve stimulation* Acta physiol scand 1964 62 137—149 — The amplitude of the uterine contraction elicited by a few seconds stimulation of the hypogastric nerve depends on (1) the stimulus frequency the contraction being maximal at 30 to 50 cps (2) the excitability of the myometrium being increased in a period from 20 to 60 sec following a preceding stimulus and (3) the degree of uterine distension When each stimulation for a few seconds elicits a single contraction prolonged stimulation (for several minutes) at low frequencies (5—10 cps) evokes a series of rhythmic contractions Prolonged stimulation at frequencies above 10 cps causes in addition a transient elevation of the minimum pressure I v administration of cocaine does not potentiate the response to hypogastric nerve stimulation whereas potentiation by cocaine is obtained when the agent are applied intra uterinely This effect as well as the response to adrenergic blocking agents indicate the presence of adrenergic fibres in the hypogastric nerve supplying the myometrium Evidence for cholinergic fibres to uterus in the hypogastric nerve was not obtained The uterine response to splanchnic nerve stimulation differs from the response to hypogastric nerve stimulation by a longer latency and by a more gradual onset

Electrical stimulation of the hypogastric nerve in estrogenized rabbits elicits a contraction of the myometrium usually followed by inhibition of the spontaneous rhythmic activity (Langley and Anderson 1895 Cushny 1906 Rudolph and Ivy 1930 Schofield 1959) These experiments were performed either with the isotonic recording technique or the response was studied merely by inspection However the isotonic recording technique has been shown to be a less reliable measure of myometrial contractions than is the isometric method (Csapo 1954 Setekleiv 1964 a) In any study which attempts a quantitative analysis of the response such as for instance a determination of the optimum stimulus frequency the isometric method should be used Therefore in the present study the uterine response to hypogastric nerve stimulation at various frequencies has been re investigated using isometric recording *in vivo*

In previous studies the stimulus has been applied for a few seconds only. During the present experiments it was disclosed that prolonged stimulation (several min) resulted in even more pronounced difference in the uterine response to various frequencies. Further the uterine response to hypogastric nerve stimulation was found to be conditioned by a preceding period of stimulation and finally to depend upon the degree of distension.

The nature of the transmitter released from hypogastric nerve fibres supplying the uterus has been a matter of dispute. The similarity between the uterine response to hypogastric nerve stimulation and to i. v. administration of adrenaline or noradrenaline has been taken as evidence that this nerve contains adrenergic fibres (Dale 1906, Cushny 1906, Schofield 1952, Cross 1958). Furthermore adrenergic blocking agents abolish the nerve mediated response (Schofield 1952). However potentiation of the response by cocaine has not been demonstrated *in vivo* (Labate 1941, Schofield 1952) and atropine in large doses has been shown to depress the response to hypogastric nerve stimulation (Reynolds and Foster 1941, Labate and Sheehan 1943). It has been argued that this atropine depression may be due to a direct effect on the myometrium since it has not been possible to increase the response by cholinergic potentiating agents *in vivo* (Schofield 1952). However, in the isolated rabbit hypogastric nerve — uterus preparation Varagic (1955) observed potentiation of the response to hypogastric nerve stimulation by eserine as well as by cocaine and depression of the response by atropine in low concentrations. He concluded that the hypogastric nerve contains both adrenergic and cholinergic excitatory fibres. The effects of some autonomic agents were reinvestigated in the present study using the isometric recording method.

The uterine response to hypogastric nerve stimulation and to adrenergic transmitters indicates that the myometrium may be under the influence of the sympathico-adrenal system. It is of importance when activating this system either reflexly or by stimulation of central nervous structures to separate the direct nervous influence through the hypogastric nerve and the hormonal discharge of the adrenal medulla. With the purpose of studying these differences the responses to hypogastric and splanchnic nerve stimulation respectively has been compared.

Material and methods

The results are based on data gained from a total of twenty nine rabbits (weight 2.5—4.1 kg). The animals were ovariectomized and treated with diethylstilbestrol (0.5 mg every second day) for at least one week before the experiment.

The *operative procedures* and the *technique used for isotonic and isometric recording* of the uterine contractions have been described elsewhere (Setekleiv 1964 a).

Anesthesia. The rabbits were anesthetized with a 1 per cent solution of chloralose (40 mg/kg) and a 25 per cent solution of urethane (500 mg/kg), one half given i. v. and the rest i. m.

Stimulation of the hypogastric and splanchnic nerves. The hypogastric nerve was scrutinized on the aorta above the bifurcation by lifting the uterus and the rectum. The nerve was sectioned just above the aortic bifurcation. The splanchnic nerves on the left side were sectioned just beneath the diaphragm through a midline incision extending as high up as to the processus xiphoides. In some cases the left splanchnic nerves were reached by a retroperitoneal approach through an about 5 cm incision in the flank. The peripheral end of the nerve to be stimulated was thread through a polyethylene tube with two circular silver electrodes embedded on its inner wall about 5 mm apart. The nerve was tied to the proximal end of the tube which was filled with mineral oil at 39° C. For electrical stimulation square wave pulses of 1 msec duration were usually delivered. The stimulus could be varied with regard to pulse duration, intensity and frequency. Supramaximal intensities were always employed.

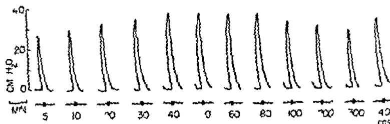


Fig 1 Uterine response to electrical stimulation of the hypogastric nerve at frequencies varying from 5 to 300 cps for 5 sec (15 V 1 msec) Isometric recording (Exp 148)



Fig 2 Duration of the uterine response (contraction — inhibitory period) to hypogastric nerve stimulation at arrows Maximal response at 30 cps (15 V 1 msec 5 sec) Isotonic recording (Exp 48)

Results

I Stimulation of the hypogastric nerve

When the uterus had regained its activity after the operation as indicated by the presence of stable rhythmic contractions stimulation of the hypogastric nerve elicited a contraction the latency of which was usually between 3 and 4 sec with variations from 2 to 6 sec The following observations are based on data in twenty two animals

a Stimulation frequency

Short lasting stimulation The myometrial response to a few sec stimulation of the hypogastric nerve consisted of a contraction followed by a decrease or an absence of the rhythmic activity When stimulating with a constant voltage supramaximal frequency 30 cps and pulse duration 1 msec and increasing periods of stimulation optimal responses were obtained at stimulation periods of about 4 sec

Fig 1 illustrates the uterine response to hypogastric nerve stimulation at increasing frequencies 5—300 cps for 5 sec Maximum response was in this case obtained at frequencies above 40 cps In other animals maximum responses were reached at frequencies between 30 and 50 cps At stimulation frequencies above 100 cps the amplitude decreased When again stimulated at optimum frequency 40 cps a greater contraction was elicited The amplitude however does not reach the peak pressure of the former contraction at the same frequency suggesting an exhaustion of the preparation after repeated stimulation

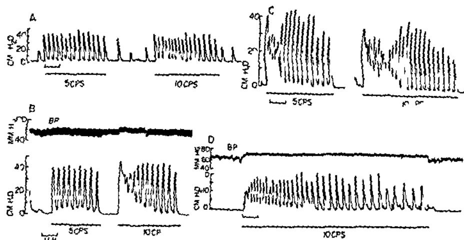


Fig 3 Uterine response to various frequencies by prolonged stimulation of the hypogastric nerve A frequency of 5 cps elicits either a series of contractions (A) or in addition an increase in minimum pressure (B) The latter is always seen after 10 cps (A—D) Elevation of blood pressure during stimulation (B and D) A Exp 113 B Exp 139 C Exp 115 and D Exp 112

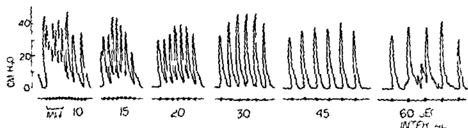


Fig 4 Potentiation of the uterine response to hypogastric nerve stimulation for 5 sec every 15 20 30 45 and 60 sec respectively (5 V 30 cps 1 msec) (Exp 111)

Similar results were obtained with the isotonic recording technique when the duration of the response was taken as a measure of the effect In the experiments illustrated in Fig 2 the amplitude of the contractions was maximal at frequencies from 20 to 50 cps However if the total duration of the contraction plus the following inhibitory period is considered this was found to vary with the frequency of stimulation maximal duration being recorded at a frequency of 30 cps

Prolonged stimulation Stimulation at 1 cps for several min usually gave no effect but sometimes a slight elevation of the intra uterine pressure was encountered in uteri with weak rhythmic activity On the other hand in uteri with vigorous spontaneous contractions a slight decrease in amplitude of the rhythmic activity ensued

The response to stimulation at 5 cps usually consisted of a series of contractions with a rather constant amplitude and frequency and with no or only a slight elevation of the minimum pressure (Fig 3 A and B) In some animals stimulation at 5 cps resulted in an initial strong contraction followed by a series of smaller contractions (Fig 3 C) In these cases the minimum pressure increased initially and then declined to reach

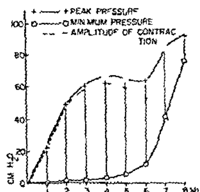


Fig 5 Effects of distension on the uterine response to hypogastric nerve stimulation (7 V 30 cps 1 msec 5 sec) The uterus was distended in steps of 1 ml from 1 to 8 ml The minimum pressure (0 — 0) increases at first slowly at higher degrees of d tens on more steeply (above 6 ml) The amplitude (— — —) of the response to hypogastric nerve timulation ncreases up to a maximum (4 ml) and then decreases Since the rapid increase in m nimum pressure occurs at h gher distens on a d p on the peak pressure (+ — +) is produced (Exp 13a)

nearly zero after about 1 min The contractions then again increased in amplitude and a relatively high peak pressure was maintained for a minute or two The frequency of the contractions decreased in the course of the continuous stimulation

On stimulation at higher frequencies (10—100 cps) there was always an enhancement of the minimum pressure its height and duration increasing with increasing stimulus frequencies The amplitude of the contractions was reduced giving the total response a more tonic appearance At the highest frequencies used the induced contractions were less sustained In some experiments the initial contraction was small or absent although an elevat on of the minimum pressure occurred (Fig 3 D)

Hypogastric nerve stimulation was ordinarily accompanied by a slight elevation of blood pressure (Fig 3 C and D)

These findings indicate that although stimulation at frequencies of 30—50 cps elicit an optimal single response the myometrium tolerates best low frequency activation

(b) Repetitive stimulation

The responses of the uterus to stimulation of the hypogastric nerve with constant stimulus parameters were often variable The influence of variation of the interval between successive stimulations on the uterine response was studied in five animals in which the hypogastric nerve was stimulated with trains of pulses (supermaximal intensity 30 cps 1 msec 5 sec) Each train produced a single contraction The intervals between the train in each series varied from 10 to 90 sec At high repetition rates (10 and 15 sec between each train) a tetanic contraction ensued with incomplete relaxation after each single contraction and elevation of the minimum pressure Fig 4) At lower repetition rates (20 to 60 sec between each train of pulses) there usally was a greater or comp lete relaxation of the myometrium between each stimulat on During the f rst 3 to 6 st mulations there was a potentiation of the response this was present up to 60 sec intervals betw een the trains and was absent at 90 ec ntervals After 10—12 stimulations the response gradually decreased in each ser es

(c Influen e of uterine distension

The degree of uterine distension greatly influen ed the esponse to qui k distension as well as the rhythm c spontaneous activity Set kler 1964 b Thi has also been

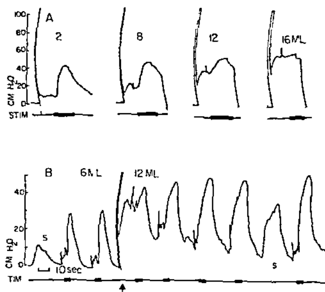


Fig 6 A The degree of myometrial activation during the early stretch response compared with the maximal myometrial contraction in response to hypogastric nerve stimulation (7 V 30 cps 1 msec) Quick distension with 2 ml Tyrode solution elicits no early stretch response Submaximal myometrial activation by injection of 8 and 12 ml complete activation by 16 ml *B* Effects of quick distension (6 ml) on the response to hypogastric nerve stimulation (7 V 30 cps 1 msec 5 sec) During and immediately after the early stretch response the contractions are smaller than when the uterus has adapted to the new volume s spontaneous contraction (Exp 143)

shown to determine the effects of hypogastric nerve stimulation In four animals the uterine response to such stimulation (supramaximal intensity 30 cps 1 msec 5 sec) was studied during successive increase in the intra uterine pressure in steps of 1 ml The fluid was not withdrawn between the injections A representative experiment is given in Fig 5 The minimum pressure obtained after each injection and the peak presure of the response to hypogastric nerve stimulation have been plotted against the intra uterine volume At higher volumes (in this case at 4 ml and above) the injection elicited a contraction the early stretch response (Setekleiv 1964 b) When the uterus had relaxed after this response the hypogastric nerve was stimulated Fig 5 shows that the amplitude of the contraction in response to hypogastric nerve stimulation increased with increasing volume up to 4 ml at higher volumes the amplitude was reduced

The influence of the early stretch response on the response to hypogastric nerve stimulation was studied in four animals During the early stretch response it was possible to excite the uterus additionally by hypogastric nerve stimulation except under a high degree of distension (Fig 6 A) In the succeeding phase corresponding to the phase of rapid decrease in minimum pressure (Setekleiv 1964 b) the responses to hypogastric nerve stimulation were relatively small in the second phase of slower decrease in minimum pressure, the responses increased until a relatively stable level was reached (Fig 6 B) Thus the response to hypogastric nerve stimulation behaves in a similar

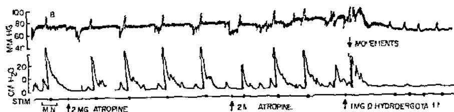


Fig 7 Effects of 2 + 2 mg atropine (1 48 mg/kg) on blood pressure and uterine response to hypogastric nerve stimulation (6 V 30 cps 1 msec 5 sec) Transitional depressive effect only is obtained Sustained blood pressure drop and nearly complete abolition of the uterine response after injection of 1 mg d hydroergotamine (0 37 mg kg) (Exp 111)

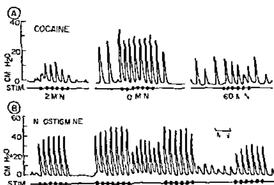


Fig 8 A Potentiation of the uterine response to hypogastric nerve stimulation (6 V 30 cps 1 msec 5 sec) after intra uterine application of 0 1 per cent cocaine First series of stimulation 2 min after cocaine instillation the second 20 min and the third 60 min after instillation After 60 min the responses is reduced due to the local anesthetic effect B Potentiation of the uterine response to hypogastric nerve stimulation and of the rhythmic activity by i v administration of 0 1 mg/kg neostigmine (Exp 137)

manner as the amplitude of the rhythmic contractions after a quick distension (Setekleiv 1964 b)

The relaxation between successive maximal contractions of hypogastric nerve stimulation was more pronounced than between successive rhythmic contractions

(d) Effects of autonomic agents

Autonomic blocking agents — *Atropine* was given to three animals In the experiment shown in Fig 7 atropine was injected in the saphenous vein in doses of 2 mg (0 74 mg/kg) The hypogastric nerve was stimulated for 5 sec (supramaximal intensity 30 cps 1 msec) The injection of atropine resulted in a transient fall in blood pressure and a slight initial reduction of the amplitude of the response to hypogastric stimulation The effects were more marked after the second injection of 2 mg of atropine but again the effect was transient and after 3 min the response had nearly reached its original value

Dihydroergotamine In the same animal referred to above (Fig 7) 1 ml of Dihydroergotamin® Sandoz (0 37 mg/kg) was given i v This resulted in a sustained decrease of blood pressure and an almost complete abolition of the response to hypogastric nerve stimulation *Regitin®* Ciba in doses of 10 mg (3 7—4 mg kg) i v similarly completely blocked the response to hypogastric nerve stimulation (three animals)

Autonomic potentiating agents — Small doses of *cocaine* (1—3 mg) administered i v did not potentiate the response to hypogastric nerve stimulation A slight decrease

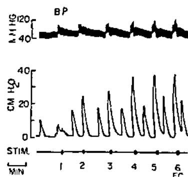


Fig 9 Blood pressure and uterine responses to splanchnic nerve stimulation for periods of 1 to 6 sec (8 V 20 cps 1 msec) (Exp 140)

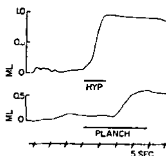


Fig 10 Latency of uterine response to hypogastric and splanchnic nerve stimulation (Exp 49 and 50)

the spontaneous rhythmic activity as well as in the response to hypogastric nerve stimulation was often observed To avoid the general effect cocaine was added to the intra uterine fluid Using solutions of 0 01 per cent the response to hypogastric nerve stimulation as well as the spontaneous rhythmic activity increased initially In the experiment shown in Fig 8 the response is increased 20 min after the application of cocaine Later on however the response decreased again probably due to the local anesthetic effect of cocaine Similar results were obtained after intra uterine application of 0 1 per cent lidocaine (Xylocain^(R) Astra) Potentiation of the response to hypogastric nerve stimulation was also seen after higher concentrations of cocaine and Xylocain (0 5—2 per cent) but then the local anesthetic effect appeared earlier

Neostigmine I v administration of 0 1 mg/kg neostigmine potentiated the effect of hypogastric nerve stimulation (Fig 9) Twitches of the skeletal muscles and excessive salivation occurred simultaneously the pupils were dilated and the amplitude of the respiratory movements were increased Later on the rhythmic activity as well as the response to hypogastric nerve stimulation gradually decreased apparently due to deterioration of the preparation

2 Stimulation of the splanchnic nerve

The myometrial response to splanchnic nerve stimulation was studied with the purpose to reveal any difference between the responses to stimulation of the splanchnic and hypogastric nerves

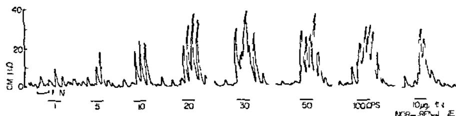


Fig 11 Uterine response to splanchnic nerve stimulation for 1 min at frequencies of 1 5 10 20 30 50 and 100 cps (8 V 1 msec) Note that the initial strong contraction seen in the uterine response to hypogastric nerve stimulation is absent At higher frequencies increase in minimum pressure For comparison the response to 10 µg noradrenaline administered by continuous i v injection in 1 min is shown (right) (Exp 104)

In three animals the splanchnic nerve was stimulated for periods of increasing duration and with the other stimulus parameters constant (supramaximal intensity 30 cps 1 msec) It was found that stimulation periods varying from 4 to 10 sec were required to elicit a maximal uterine response

The latency Fig 10 shows a comparison of the uterine response to hypogastric and splanchnic nerve stimulation The latencies are about 3 and 10 sec respectively The latter value varied between 10 and 15 sec in the various preparations

Stimulation frequency In the experiment shown in Fig 11 the splanchnic nerve was intermittently stimulated for 1 min at frequencies varying from 1 to 100 cps The response to 1 cps is minimal At 5 cps two contractions are seen the second stronger than the first one At increasing rates of stimulation up to 30 cps the response is augmented At frequencies above 15 cps some elevation of the minimum pressure was encountered The latency decreased from 25 sec at 5 cps to 13 and 14 sec at 20 and 30 cps respectively At 10 50 and 100 cps the latency was 15 sec

In order to make some estimate of the amount of the adrenal medullary discharge 10 µg noradrenaline was administered into the saphenous vein by a motordriven syringe for continuous injection The injection time was the same as the stimulation period 1 min As can be seen from Fig 11 the uterine response to splanchnic stimulation at optimal frequency exceeded the response to this dose of noradrenaline

Prolonged stimulation of the splanchnic nerve at a stimulus frequency of 5 cps (several min) resulted in a series of contractions At higher frequencies the minimum pressure was elevated Thus the same features with regard to the contractions and the minimum pressure of the uterine response appeared after splanchnic as was found after hypogastric nerve stimulation

The response to splanchnic nerve stimulation was blocked by adrenalectomy

Discussion

The myometrial response to hypogastric nerve stimulation

The present investigation has confirmed that electrical stimulation of the hypogastric nerve elicits a contraction of the uterus in estrogenized rabbits The long latency of 2—6 sec may in part be due to the recording technique By the method used the whole

or a relatively large part of the uterus must contract to develop a rise in the intra uterine pressure By inspection of the exposed uterus it can be seen that the steep pressure increase after hypogastric nerve stimulation is caused by a simultaneous contraction of the whole uterus

To obtain maximal contraction it was necessary to stimulate the hypogastric nerve at optimum frequency for at least 4 sec This duration is the same as that required on direct electrical stimulation of the uterus when stimulated with optimum frequency (Csapo 1954 Schofield 1954 1955)

The optimum stimulus frequency was found to be 30 to 50 cps which is similar to the value of 50 cps determined by Schofield (1952) who used isotonic recording However in isotonic recordings the amplitude of the contractions may be maximal in spite of partial myometrial activation To get a true picture of the isotonic response it is therefore necessary to take into account also the duration of the response

The mechanisms underlying the varying response to different frequencies has recently been elucidated by Burnstock and Holman (1961) By intracellular recording in another adrenergic effector organ the vas deferens of the guinea pig they found that the rate of depolarization reached its maximum with optimum spike generation at about 50 cps Since the tension developed in smooth muscles depends on the frequency of the spike discharge (Bulbring 1955 Holman 1958) maximal contraction is obtained when the spike discharge is optimal

The potentiation of the uterine response to repeated stimulation of the hypogastric nerve has not been described previously In sympathetic ganglia a period of increased responsivenes after repetitive stimulation has been reported by several investigators (Larrabee and Bronk 1947 Eccles 1952) Post activation potentiation of gastric and intestinal contraction in response to vagus nerve stimulation was obtained by Blair *et al* (1959) By direct stimulation of the myometrium an increased response or a positive staircase has been obtained after repetitive stimulation of the uterus *in vitro* (Csapo and Corner 1952) The positive staircase was found to be characteristic of the estrogene treated uterus while a negative staircase appeared in the progesterone dominated organ Similar characteristics according to the hormonal dominance has been found by direct stimulation of the uterus *in situ* (Schofield 1954 1955 Bengtsson 1957) It has been postulated that the potentiation is due to increased output of transmitter substance caused by presynaptic hyperpolarization (Eccles 1957)

The increase in minimum pressure at high frequency stimulation (Fig 3 and 4) may be due to a tetanus with activation of the myometrium before it is completely relaxed after the preceding contraction It has however also been proposed that stimulation of the myometrium may activate two different contractile systems a tonic one caused by membrane depolarization and a tetanic one due to spike activity (Jung 1961) It is not possible from the result of the present study to decide whether the increased minimum pressure is due to fusion of contractions or to activation of a tonic system

Another question is why the minimum pressure is not maintained but decreases relatively steeply during continuous high frequency stimulation of the hypogastric nerve The reason for this decline may be (i) either a decreased output of the transmitter (ii) a change in the excitability of the myometrium or (iii) mechanical factors in the myometrium If these alternatives are considered further

(i) The content of transmitter in sympathetic nerves is not exhausted by stimulation for relatively long periods (Luco and Goni 1948) Brown Davies and Gillespie (1958) found that the transmitter content in the venous blood from the intestines in cats in

creased to a steady level lasting the whole period of sympathetic stimulation (up to 100 sec) The plateau level was reached sooner at higher frequencies of stimulation Repeated stimulation leads to diminution in transmitter output. However the transmitter content in the venous blood might be regarded as an overflow due to an unphysiological stimulation frequency (Folkow 1952) and it does not tell if sufficient concentrations of transmitter reach the receptor site On the other hand in the present study it was regularly found that the uterine response decreased after repeated stimulation especially after high frequencies and prolonged stimulation (Fig 1)

(ii) The frequency of the contractions diminishes during the decline in minimum pressure This might be taken as an indication of decreased excitability of the myometrium but this decline most likely is caused by the decrease in minimum pressure *per se* (Setekleiv 1963 b)

(iii) There was a higher degree of relaxation between successive maximal responses to hypogastric nerve stimulation than between the submaximal rhythmic contractions (Fig 6) The contractions influence the viscous elements in the myometrium the muscle cells elongate and loose tension a process possibly facilitated by the transmitter (Winton 1930) Most likely the decrease in minimum pressure is mainly due to these mechanical properties of the myometrium

In this connection it should be mentioned that it is not known how the myometrium is affected by circulatory changes induced by the hypogastric nerve stimulation

The size of the response to hypogastric nerve stimulation depends also on the degree of uterine distension Moderate degrees of distension favour the contractions whereas the response is reduced at greater distension These variations are principally similar to those described by the length tension diagram of other kinds of muscles

The effect of hypogastric nerve stimulation during the early stretch response indicates that the same muscular cells are involved in the responses to distension and to nervous stimulation This is also substantiated by the similarity of the responses to distension and to hypogastric nerve stimulation during the rapid and slow phases of decrease in minimum pressure

The uterine contractions can be driven unchanged for longer periods only at low frequency nerve stimulation when the minimum pressure is not increased At higher stimulation frequencies the rate and amplitude of the contractions decrease proportionally with the increase in frequency of stimulation.

During physiological conditions the uterus is probably stimulated with nervous impulses at low frequencies only By recording the discharge in sympathetic nerves it has been found that the frequency seldom exceed 10—20 cps (Bronk *et al* 1936)

The transmitter released by hypogastric nerve stimulation

As mentioned previous investigators have failed to obtain potentiation of the response to hypogastric nerve stimulation by i v administration of cocaine *in vivo* (Labate 1941 Schofield 1952) In the present study it was found that i v injection of cocaine rather depressed the uterine motility A slight depression was also observed by i v administration of small doses of adrenaline and noradrenaline (Setekleiv 1964 a However intra uterine application of small concentrations of cocaine and Xylocain potentiated initially the response to hypogastric nerve stimulation until the local anesthetic effect of these drugs dominated Therefore when the general effects of cocaine was avoided this agent potentiated the uterine response to hypogastric nerve stimulation This result together with the fact that the adrenergic blocking agents dihydroergotamine

and Regitin blocked the myometrial response strongly indicates that adrenergic fibres are present in the hypogastric nerves to the myometrium.

Regarding the question of cholinergic fibres in the hypogastric nerve in addition to the adrenergic ones atropine was found in the present study to depress the response to hypogastric nerve stimulation However the effect was transitional and can be explained as an effect directly on the myometrium The minor fall in blood pressure does not influence the response The interpretation of the potentiating effect of neostigmine is also difficult Acethylcholine applied intra uterinely similarly increases the rhythmic activity and the sensitivity of the uterus (Setekleiv 1964 b) The augmented response is therefore not necessarily due to potentiation of cholinergic fibres in the hypogastric nerve It can be caused by an overflow of acetylcholine due to the neostigmine administration

It may also be due to accumulation of local non nervous acetylcholine (Burn 1950) However neostigmine applied intra uterinely had only little or no potentiating effect.

Burn and Rand (1962) have postulated that the release of the adrenergic transmitter is initiated by acetylcholine Since the uterine response to acetylcholine differs from the response to adrenaline it seems most likely that the potentiation obtained after neostigmine can be explained as reduced destruction of acetylcholine

The myometrial response to splanchnic nerve stimulation

The response to splanchnic nerve stimulation differs from that to hypogastric nerve stimulation in two particular ways viz with regard to latency and to the onset of the response The latency on splanchnic stimulation was more than 10 sec as compared with 2 –6 sec after hypogastric stimulation In activation of the sympathico-adrenal system, either reflexly or by stimulation of central nervous structures a latency less than 10 sec would indicate that at least the first part of the response must be due to stimulus mediated through the nervous supply to uterus

The pattern of onset of the response is more slowly and consists of several contractions with increasing amplitude as compared to the initial strong contraction after hypogastric nerve stimulation. The uterine excitation by adrenal medullary discharge may be regarded as more physiological than that of hypogastric stimulation at high frequency Only at hypogastric stimulation at low frequency (5 cps) a similar response as splanchnic stimulation was obtained Since discharge rate in sympathetic nerves is low (Bronk *et al* 1936) the uterus most likely is activated in this manner during physiological conditions

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Effect of Ethionine on the Incorporation of Palmitic Acid-1-C^{14} into Rat Serum Lipoproteins

By

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Abstract

Kärvinen, E. O. Koskimies and M. Miettinen. *Effect of ethionine on the incorporation of palmitic acid 1-C^{14} into rat serum lipoproteins* Acta physiol. scand 1964 62 150—155 — Ethionine administration to female rats had no significant effect on the radioactivity of the serum chylomicron fraction after ingestion of palmitic acid 1-C^{14} On the other hand ethionine administration resulted in a decrease in the incorporation of palmitic acid 1-C^{14} into the serum albumin plus α lipoprotein fraction and into the α lipoprotein fraction There was no significant difference in the activity of the serum β lipoprotein fraction between the ethionine and control rats. Total liver fat and the total amount of palmitic acid 1-C^{14} incorporated in the liver fat were significantly higher in the ethionine treated animals than in the saline controls However the relative specific activity of the liver fat of the ethionine treated group was lower than that of the controls.

It has been reported by Borgström et al. 1961 that rats with ethionine induced fatty livers remove injected chylomicrons with a slower rate than do normal rats Ethionine administration has also been shown to increase the radioactivity in the chylomicron fraction above the level found in normal rats after oral administration of cholesterol-4-C^{14} (Kärvinen and Miettinen 1962 Ethionine administration is further known to lower serum phospholipids and fatty acids as well as high and low density lipoproteins in the dog (Feinberg et al. 1954 Wang et al. 1958 In the rat, ethionine administration was found to produce a marked decrease in the incorporation of radioactivity into $\alpha_1$lipoprotein after ingestion of cholesterol-4 C^{14} whereas there were no significant differences in the activities of the α and β lipoprotein fractions between the ethionine and control rats (Kärvinen and Miettinen 1962

In the present paper effects of ethionine on the incorporation of ingested palmitic acid 1 C^{14} into rat serum protein fractions are reported.

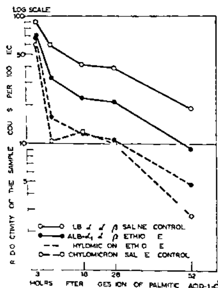


Fig 1

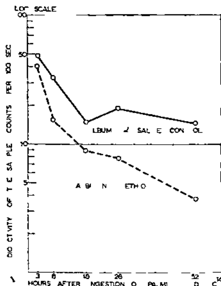


Fig 2

Fig 1 Semilogarithmic plot of the radioactivities of albumin + lipoprotein and chylomicron fractions in ethionine and control rats after ingestion of palmitic acid 1 C

Fig 2 Semilogarithmic plot of the radioactivities of albumin + α lipoprotein fraction in ethionine and control rats after ingestion of palmitic acid 1 C

Material and methods

Female rats of the Wistar strain weighing 200 to 250 g were caged individually and given a stock diet for 3 weeks ad libitum During the experiment the rats were fed with entire wheat flour and water

On the first and second experimental day the ethionine rats were given daily three i p injections of 35 mg of DL-ethionine dissolved in 2 ml of saline at 9 AM 3 PM and 10 PM On the third and fourth day two i p injections of 35 mg of ethionine were given daily at 9 AM and 3 PM On the fifth day 35 mg of ethionine were given i p at 9 AM

The saline rats were given i p injections of 2 ml of saline according to the same schedule

Between the first and second day all rats were fasted overnight and fed on the second day at 1100 hours 4 μc of palmitic acid 1 C[14] in 2g of entire wheat flour The labeled palmitic acid was supplied by the Radiochemical Centre Amersham England Palmitic acid 1 C[14] was dissolved in ether and poured in the dry entire-wheat flour Then the dry diet was made to a paste by adding water The rat ate the paste in about 15 min

From 7 ethionine rats and 9 saline rats blood samples of about 1 ml were taken under ether anesthesia from the tail 3 8 18 and 28 hours after the ingestion of the labeled palmitic acid From 5 ethionine rats and 6 saline rats the blood samples were taken 28 52 and 76 hours after the ingestion of the labeled palmitic acid

Fractionation of the serum lipoproteins was carried out according to the paper electrophoretic method of Nikkila (1953) as modified and described elsewhere (Miettinen 1957)

The electrophoresis paper was cut into stripes containing albumin plus globulin plus α lipoprotein α globulin plus α lipoprotein β globulin plus β lipoprotein and the area around the starting line containing chylomicrons The lipids were extracted and counted for radioactivity as reported in the following

Five ethionine and 8 saline rats were killed 8 hours after the ingestion of the labeled palmitic acid The livers were excised and immersed in ethanol The samples were then boiled with 10

TABLE I Mean radioactivities contained in the lipid of the serum l poprotein fractions of th ethionine and saline rats The values are expressed in counts per 100 sec per 0 5 ml of serum

Time (hrs)	Fraction	Mean radioactivity counts/100 s c Saline rats	Ethionine rats	P
		n = 9	n = 7	
8	Albumin + α	32 8	1ɔ ɔ	0 0ɔ
	α	13 5	7 1	
	β	13 5	10 1	
	Chylomicron	10 7	16 1	
		n = 8	n = 7	
18	Albumin — α	14 4	9 0	0 0ɔ
	α	13 6	6 3	0 0ɔ
	β	13 2	7 ɔ	
	Chylomicron	12 0	1? 6	
		n = 14	n - 10	
?8	Albumin + α	18 4	7 8	0 0ɔ
	α	12 1	6 4	0 0ɔ
	β	9 1	6 8	
	Chylomicron	10 1	10 1	
		n = 6	n = 5	
ɔ2	Albumin + α	12 2	3 8	
	α	3 6	2 1	
	β	3 9	3 1	
	Chylomicron	2 7	4 7	

TABLE II Radioactivity of the liver fat in the ethionine and saline rats 28 hours aft r ing stion of palmit c acid 1 C (mean ± standard deviation)

	Number	Total liver fat (mg)	Total radioactivity in liver fat counts/100 sec	Relative specific activity of liver fat
Ethionine rats	5	1 058±42,	19 498±4 ?10	?0 1±ɔ 7
Saline rats	8	145±39	6 99F±866	ɔ0 3±10 ɔ
P value of difference		t =ɔ 27 p<0 001	t_1 =7 21 p<0 001	t 6 3ɔ p<0 001

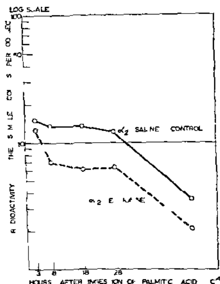


Fig 3

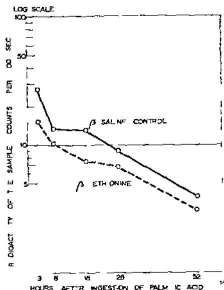


Fig 4

Fig 3 Semilogarithmic plot of the radioactivities of lipoprotein fraction in ethionine and control rats after ingestion of palmitic acid 1 C

Fig 4 Semilogarithmic plot of the radioactivities of β lipoprotein fraction in ethionine and control rats after ingestion of palmitic acid 1 C

ethanolic KOH for 2 hours The mixture was then acidified by adding H_2SO and the acidified mixture was allowed to stand overnight Then the lipids were taken up in petroleum ether and washed with 50 ethanol and with water Samples of the lipid were then plated on steel planchets and counted The counts were corrected for mass absorption.

Results

In Fig 1 the total radioactivities of the chylomicron fraction at various interval are shown There was no significant difference in the activity of the chylomicron fraction between the saline controls and the ethionine group (Table I) As is shown in Fig 1 there seems to be a constantly higher level of radioactivity bound in the total lipoprotein plus albumin of the saline controls as compared with the ethionine treated animals

It is seen from Table I and Fig 2 that ethionine administration to female rats led to a decrease in radioactivity found in the lipid of the albumin plus α_1 lipoprotein fraction after giving palmitic acid 1 C^{14} orally The difference between ethionine and saline rats was significant 8 18 and 28 hours after palmitic acid 1 C^{14} ingestion

It is evident from Table I and Fig 3 that administration of ethionine resulted in a significant decrease in the radioactivity of the α lipoprotein fraction when compared with the saline group

In Fig 4 the radioactivities of the β lipoprotein fraction are shown There was no significant difference in the activity of the β lipoprotein fraction between the ethionine and saline animals (Table I)

The radioactivity of the liver fat in rats killed 28 hours after ingestion of labeled palmitic acid was in the ethionine group about three times as high as in the saline group. The difference was statistically significant (Table II).

Total liver fat in the ethionine group averaged 1058 mg and in the saline group 145 mg. The difference was statistically significant. However, the relative specific activity of the liver fat of the ethionine animals was lower than that of the saline controls.

Discussion

The present results showed no significant difference in the activity of the chylomicron fraction between the ethionine and saline rats after ingestion of palmitic acid 1 C^{14}. On the other hand, absorption of palmitic acid 1 C^{14} from the gastrointestinal tract of ethionine treated rats has been found by us (Karvinen and Miettinen 1963) to be significantly lower than in saline rats. Thus it seems that the disappearance of the chylomicron label from the circulation is impaired in the ethionine treated animals. This is in accordance with the finding of Borgstrom et al. who injected chylomicrons labeled with palmitic acid 1 C^{14} and noted a decreased rate of removal of the chylomicrons in the rats with ethionine induced fatty livers.

In the present study the total amount of radioactivity remaining in the liver 28 hours after the ingestion of labeled palmitic acid was found to be significantly higher in the ethionine treated animals than in the saline group. Since the inflow of labeled chylomicrons into the livers of the ethionine rats however may not be higher than in the saline rats, the hepatic accumulation of the label in the ethionine animals may be taken to indicate an impairment in the removal of the palmitic acid from the liver. After an injection of palmitic acid 1 C^{14} bound to albumin in normal and ethionine treated rats more of the label was recovered by Olivecrona (1962a) in the livers of the ethionine than of the normal rats. He concludes that the accumulation of neutral fatty acids in the livers of the ethionine treated rats appears at least in part to be due to decreased transport of fatty acids from the liver in the form of plasma triglyceride fatty acids.

It was noted by Olivecrona (1962b) that all the classes of lipids carried by the lipoproteins (i.e. phospholipids, cholesterol esters, free cholesterol and glycerides) were depressed in ethionine treated rats. Robinson and Harris (1961) have shown that ethionine administration markedly reduces the incorporation of C^{14} leucine into serum lipoproteins. These findings suggest that the synthesis of serum lipoproteins is depressed in the ethionine treated rats.

In an earlier work from this laboratory Karvinen and Miettinen (1962) have been able to show that ethionine administration to rats results in a marked decrease in the incorporation of radioactivity into the α_1 lipoprotein fraction after ingestion of cholesterol-4 C^{14}. In the present study the incorporation of palmitic acid 1 C^{14} into the albumin plus α_1 lipoprotein fraction was significantly depressed in the ethionine treated rats. This finding may suggest that the synthesis of the α_1 lipoprotein is depressed under the influence of ethionine since in the saline rats the uptake of palmitic acid 1 C^{14} activity into the albumin plus α_1 lipoprotein fraction seems to occur in two rather separate phases, whereas in the ethionine treated animals only the first phase (albumin) is present (Fig. 2 and Table I).

The incorporation of palmitic acid 1 C^{14} activity into the α lipoprotein fraction of the ethionine treated animals was found to be depressed in the present study. In an

earlier work by Karvinen and Miettinen (1962) on the incorporation of cholesterol 4 C^{14} into the serum lipoprotein fractions it was noted that the incorporation into the $\alpha_2$lipoprotein fraction was slightly lower in the ethionine treated animals but the difference was not statistically significant These findings seem to indicate that the synthesis of the α lipoprotein is decreased by ethionine

On the other hand the incorporation of palmitic acid 1 C^{14} into the β lipoprotein was not significantly altered in the ethionine treated rats This is in accordance with our findings (1962) on the incorporation of cholesterol 4 C^{14} into the β lipoprotein fraction

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The Distribution of Noradrenaline and Adrenaline in the Fallopian Tube of the Rabbit

By

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Abstract

Brundin, J. *The distribution of noradrenaline and adrenaline in the Fallopian tube of the rabbit.* Acta physiol. scand. 1964. 62. 156—159. — The present study was undertaken in an attempt to explain the functional difference between the isthmic and ampullary regions of the oviduct in the rabbit by a difference in the distribution of their adrenergic nerve supply. The isthmus contains considerable amounts of noradrenaline (NA) while the NA contents of the ampulla and uterus are low. Adrenaline (A) was found in low concentrations in all of the preparations studied. Some implications of the distribution of NA in the different regions are discussed.

Previous studies of the innervation of the Fallopian tube have indicated that the organ is supplied by sympathetic and parasympathetic nerve fibers (Frankenhauser 1866, Gawronsky 1894, Kok 1927). However, the exact distribution of autonomic fibers within the oviduct has not been studied. In the present investigation the contents of NA and A have been estimated in both the uterine and ovarian halves of the rabbit Fallopian tube. The NA content differs in these two parts of the oviduct and was found to be low in the uteri examined.

Materials and methods

Five groups of six albino rabbits (Swedish Land race) weighing 2.6—3.8 kg were used in this study. It was necessary to use groups of animals because of the low total amount of NA and A in a single oviduct. The animals were sacrificed one group at a time. The Fallopian tubes were dissected out, freed from the surrounding fat tissue and cut transversely into two halves of equal length. The twelve uterine and the twelve ovarian halves were stored separately at −20 C for a maximum of 48 hours, extracted with 10 trichloracetic acid and their contents of NA and A estimated according to Euler and Lishajko (1961).

The extracted NA was identified in an Aminco-Bowman spectrophotofluorimeter. The NA and A content of the uteri of some rabbits was also determined using the same technique for extraction and estimation.

TABLE I NA content ($\mu g/g$) in the uterine halves (U) and the ovarian hal es (0 of the rabbit oviduct

Group	U	0
I	1 72	0 3
II	0 43	0 12
III	1 98	0 11
IV	2 19	0 34
V	1 80	0

Results

The amount of NA in the two portions of Fallopian tube in the 5 groups of rabbits is shown in Table I The NA content in the uterine halves of the oviduct is significantly greater than in the ovarian halves Fig 1 shows the identification of the extracted NA The statistical analysis of the NA content refers to pair tests where the content of the uterine halves has been compared to that of the ovarian halves of the same group of animals ($0 01 > p > 0 002$)

The content of A in the Fallopian tubes studied was too low to be estimated with an certainty by the method used

The NA content of the rabbit uteri was determined in 3 rabbits to 0 34 0 45 and 0 53 $\mu g/g$ (average 0 44 $\mu g/g$) The A values were too low for distinct estimation

Discussion

In the rabbit the isthmus of the Fallopian tube consists of the uterine half and the ampulla and infundibulum constitute the ovarian half of the organ (Greenwald 1961 If the NA content of a tissue reflects the density of adrenergic nerve terminals in the tissue as held by Euler (1956) the results suggest that the adrenergic nerve supply in the isthmus

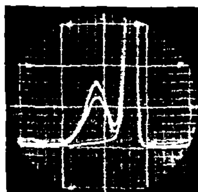


Fig 1 Fluorescence spectrum obtained in Aminco-Bowman spectrophotofluorimeter Excitation wavelength 405 mμ Maximum fluorescence at 525 mμ (uncorr) Top curve standard NA Middle curve sample Bottom curve blank

differs from that of the ampulla in the Fallopian tubes The content of NA in the ampulla did not exceed the values due to the presence of perivascular vaso-constrictor nerve endings in other tissues (Euler and Lishajko 1958) On the other hand the isthmic content was high enough to be due to a specific adrenergic innervation of that part of the Fallopian tube

A histochemical study of the distribution of adrenergic nerves in the rabbit oviduct (Brundin and Wirsén 1964) has confirmed that the isthmus and especially the tubo uterine junction contains rather higher amounts of adrenergic nerve fibers than the rest of the tissue These fibers intermingle with the circular muscle fibers of the isthmic region and have the same circular course On the contrary the ovarian half of the rabbit oviduct contains only a few adrenergic fibers adjacent to the vessels

Previous data obtained from studies on the oviducts of different species have suggested a functional difference between the isthmic and ampullary parts of the organ Thus Andersson (1927) reported that ova of the sow moved mo t rapidly through the upper third of the oviduct Burdick and Pincus (1935) described a postovulatory tube locking mechanism in mice and rabbits serving to retain the recently ovulated eggs in the ampulla for three days before they were allowed to continue down to the uterus Furthermore Alden (1942) observed a tonic contraction in the isthmus of albino rats which prevented the passage of ova through the isthmus until a certain time after ovulation A similar blocking mechanism has also been reported from studies on the cow oviduct (Black and Davis 1962) Recordings of the intraluminal pressure changes in the rabbit isthmus and ampulla have shown a difference in the pattern of their motility (Greenwald 1963) The endocrine status of the animals influenced the pressure changes obtained Moreover a preovulatory occlusive mechanism in the rabbit isthmus (Brundin 1964 b) has been interpreted as due partly to a functional block in the isthmo ampullary junction (Brundin 1964 a)

Thus the distribution of adrenergic nerves in the rabbit Fallopian tube is characteristically associated with the different functional properties of the isthmic and ampullary regions It is interesting that electrical stimulation of the autonomic nerves supplying the rabbit oviduct has verified a neuronal influence on the motility of the organ (Kok 1927)

In the few uteri examined the NA and A contents were not high enough to indicate any specific adrenergic innervation other than that of the blood vessels

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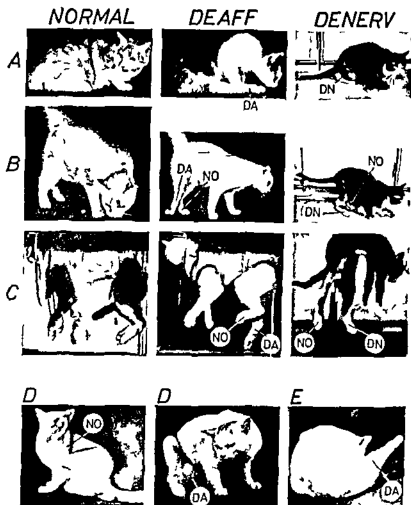


Fig 2 A Sitting B Standing C Sling position in a normal a deafferented and a denervated cat Note the extension of the deafferented hind limb (DA) absent in the normal (NO) and the denervated (DN) hind limb D Scratch reflex with the normal hind limb (NO) and the deafferented hind limb where the paw does not reach the skin E Normal performance of the cleaning reaction in a cat with a deafferented hind limb

cles of the deafferented leg did not differ from that of the non deafferented leg Similarly one year after the deafferentation the difference in weight of the calf muscles on the de afferented and the normal side in an intradurally and an extradurally operated cat did not exceed 10 % The cats did not groom the deafferented leg Ulcerations especially on the dorsum of the paw occurred in about half the animals All intradurally operated cats had transient or persistent urinary incontinence not seen in the extradurally de afferented cats

Spontaneuos activity

The first few days after operation the deafferented hind leg was flaccid in the course of 3—4 weeks the hind leg assumed a position of extension quite characteristic for the de afferented limb Extension was maintained during sitting standing stepping and jumping whether the roots were sectioned extra or intradurally In the sitting position (Fig 2A) the deafferented hind leg was held extended forward and to the side If the limb happened to come in an akward position this was not corrected The cats also often sat on the hip of the deafferented side with both legs extended to the non-deaffer ented side In the standing position (Fig 2B) the deafferented hind leg was held extend ed backwards and in slight abduction with the dorsum of the paw touching the ground the leg often slipped backwards or sidewards During stepping the flexion-extension movements occurred mostly at the hip joint the distal part of the leg being extended and the ankle joint often overextended In quick locomotion which could be as quick as in normal cats the deafferented limb was dragged behind and performed paddling like movements When the cats jumped from a table the deafferented leg was usually held forward and extended on landing the hip girdle rotated because the deafferented limb slipped on the floor To observe and record spontaneous and evoked activity in a standardized position the cats were trained to a body sling with the extremities free and off the ground (Fig 2C) In this position the deafferented leg hung lower than the nor mal leg which was usually slightly flexed at the three joints Fanning of the toes never occurred on the deafferented side Small jerky movements mostly in the proximal joints and symmetrical on the two sides occurred from time to time and were observed repeatedly in three extradurally operated cats When the cats were excited and strug gled with the three normal limbs the deafferented leg was stretched stiffly backwards Such extensor thrusts were more prominent and frequent in the intradurally deafferent ed cats

Evoked activity

Passive movements A typical supporting reaction was absent though in intradurally operated cats some active extension could be felt varying in degree from moment to moment and independent of the degree of support When the knee or ankle was bent no resistance could be felt in the extradurally operated cats and the limb dropped flac cidly as soon as it was released In the intradurally deafferented cats resistance was felt and was the same at all degrees of stretch of the extensor muscles when the limb was released it sprang back rapidly to the extended position This phenomenon was pronounced in four of the six cats whereas only little resistance was felt in two others

Cutaneous stimuli Pinching of the normal hind paw elicited varying degrees of move ments on the deafferented side in all cats It provoked a typical crossed extensor reflex only in intradurally operated cats whereas the extradurally deafferented cats showed mainly complex flexion-extension movements Other stimuli such as pinching the tail or the back of the animal elicited an extensor thrust of the intradurally deafferented hind legs such stimuli only rarely provoked a movement in legs of extradurally opera ted cats Scratching the region around the ear elicits rarely a scratch reflex in normal cats this was regularly evoked in extra and intradurally deafferented cats The scratch reflex appeared on the deafferented side three to four weeks after operation and could later be elicited on the non-deafferented side as well Two months after deaffe entation the reflex was exaggerated and could be elicited by touching the ear In the beginning the movement consisted of a stiffening of the leg followed by rhythmic flexion of the

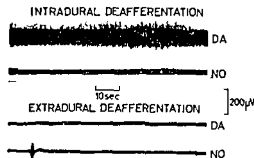


Fig 3 Spontaneous activity of calf muscles in a standardized position (sling) Note the persistent activity in the intradurally and the absence of activity in the extradurally deafferented extensor On the normal side activity is absent or occurs in small bursts

toes Later when the scratch reflex was fully developed the leg was flexed at all three joints and the rhythmic movements were most pronounced in the hip The movement pattern was the same on both sides but the anesthetic hind limb did not reach the body or neck (Fig 2D) In the initial stage of barbiturate anesthesia some cats scratched spontaneously with the deafferented leg The cleaning reaction consisting of lifting and stretching of a hind limb in response to moistening of the anal region (Gorska et al 1961) is difficult to elicit in normal cats It was seen only in two extradurally operated cats in whom it could be elicited repeatedly lifting and stretching of the deafferented hind leg was effected in a normal manner (Fig 2E)

Static reactions As in normal cats rotation and dorsi and ventroflexion of the head had no effect on the position of the hind legs When the cats were laid on their side or in the supine position both hind legs were semiflexed the deafferented hind legs of intradurally operated cats being slightly less flexed than the normal legs Hence the tonic neck and labyrinthine reflexes normally affecting the position of the fore legs more than of the hind legs were not altered after deafferentation

Position of a chronically denervated hind limb

The cat in which the right hind leg was denervated assumed a position during the different activities described above which could easily be distinguished from that of deafferented hind legs The denervated hind limb was never in a stretched position but always slightly flexed (Fig 2 A B C) No resistance could be felt on passive movements of the denervated limb

B Electromyography of spontaneous and evoked activity

Spontaneous activity

When the cats were supported in a sling with the extremities hanging freely both intradurally and extradurally deafferented hind legs hung lower than the normal legs This could be due either to active extension or to loss of normal flexion when the paw is free of the ground In intradurally deafferented extensors electromyography showed persistent activity greater in duration and amplitude than that of the normal side in extradurally deafferented limbs as in normal limbs activity in the extensors was absent or slight and when present less pronounced on the deafferented side as compared with the normal side (s Fig 3) Thus in the sling position the intradurally deafferented extremity was in a state of active extension whereas the extradurally deafferented hind leg was flaccid

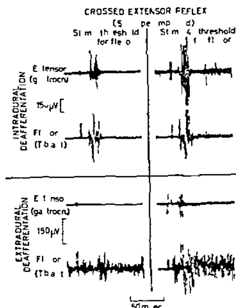


Fig 4 Activity in extensors and flexors of the deafferented hind leg elicited by electrical stimulation of the contralateral leg at the ankle (4 pulse of 0 1 msec duration) At threshold stimulation there were simultaneous bursts in the extensor and the flexor of an intradurally deafferented cat at 4 times threshold activity occurred mainly in the extensor In the extradurally deafferented cat weak and strong stimulation evoked activity mainly in the flexor

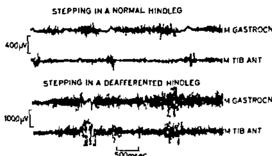


Fig 5 Electrical activity in antagonistic leg muscles during stepping Note the reciprocal innervation in a normal leg the persistent background activity and the transitory reciprocal activity followed by a phase of co-contraction in the deafferented leg

Evoked activity

In both intra and extradurally operated cats no action potentials were evoked in the deafferented muscles in response to stretch to support of the deafferented leg or to head movements Activity on scratching the ear was easy to elicit on the deafferented side the response was less pronounced and had a longer latency on the normal side

Electrical stimulation of the tibial nerve at the ankle of the normal side provoked activity which was different in the intradurally and the extradurally deafferented muscles In extradurally operated cats at stimulation strength up to four times threshold only a burst in the anterolateral leg muscles was recorded With stronger stimuli 4—8 times threshold for the flexor response) only a weak and brief burst of activity was observed in the extensors In the intradurally deafferented cats activity was elicited in the flexors as well as in the extensors at the same low threshold With stronger stimuli up to four times threshold the activity in the extensors became still more pronounced often lasting for several seconds (Fig 4)

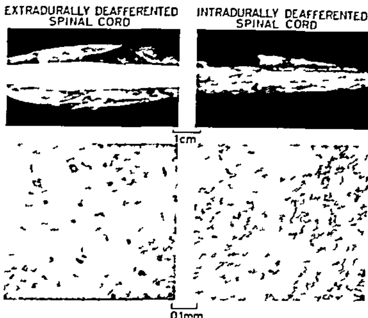


Fig 6 Macroscopic and microscopic appearance of the extradurally and intradurally deafferented lumbar regions of spinal cords. Note the deformation and adhesions present in the intradurally deafferented cord and the histological signs of damage within the grey substance.

Stepping

In cats after spinal transsection Sherrington (1910) recorded the mechanical activity of extensors and flexors during stepping movements During the extension phase the gastrocnemius muscle contracts and the anterior tibial muscle relaxes during the flexion phase the gastrocnemius relaxes and the anterior tibial muscle contracts The electromyographic findings correspond to Sherrington s analysis A grouped discharge in the extensor muscle is followed by a silent period the antagonistic flexor is activated when the flexor is inhibited Fig 5) Recording from a deafferented antagonistic muscle pair showed that the normal reciprocal innervation was disturbed Although normal reciprocal innervation could be seen, phases of simultaneous bursts in flexors and extensors were frequently observed Moreover the grouped discharges instead of being separated by silent periods occurred on a background of continuous activity (Fig 5) This disturbance in reciprocal innervation probably contributed to the extended position of the deafferented hind leg

C Anatomy of the deafferented spinal cords

Macroscopic findings The spinal cords of the intradurally deafferented cats were deformed and showed adhesions with the surrounding tissues (Fig 6) The extradurally operated spinal cords were normal except for the atrophy of the dorsal rootlets on the operated side

Microscopy On the deafferented side the spinal cords of extradurally operated animals showed degeneration in the dorsal funiculi as evidenced by the smaller size of the funic

ulus as compared with the normal side and by a cellular reaction of glial cells The intradurally deafferented cords had in addition more or less severe damage in the lateral funiculus of the operated side In two cats there were large tissue defects in the others smaller defects in the grey matter In extradurally operated cats the number of neurones was the same on the deafferented and on the non-deafferented side In the intradurally deafferented preparations there was a significant decrease in cells on the operated side The mean number of the deafferented side was 28 4 (4—6 levels) on each side of 4 cats and on the normal side 41 6 (4—6 levels on each side of 4 cats $P < 0.01\%$)

Discussion

Ranson (1928) who sectioned dorsal roots intradurally found overaction of extensor muscles This rigidity was characterized by an extended position of the deafferented hind leg in different positions by an increased resistance to passive flexion of the limb and by an exaggerated crossed extensor reflex. I found the same effect of intradural deafferentation on motility Also in the cats subjected to extradural removal of the dorsal ganglia there was overaction of extensors when the cats were sitting standing stepping or jumping However when these cats were suspended in a sling the hind limb hung loselv without resistance to passive flexion The recording of muscle action potentials in this position confirmed that in intradurally deafferented cats there was persistent activity absent in the extradurally deafferented cats Furthermore the activity in the extensors evoked by electrical stimulation of the contralateral leg was more pronounced in intradurally than in extradurally deafferented cats In both extradurally and intradurally deafferented cats normal reciprocal innervation during stepping was often replaced by co-contraction of an antagonistic muscle pair and there was no distinct inhibition between the phases of contraction Thus an overaction of extensors as well as disturbed reciprocal innervation developed within 3—4 weeks after intradural and extradural deafferentation But only intradurally operated cats showed rigidity characterized by increased resistance to passive flexion of the limb and by persistent electrical activity in extensor muscles This indicated that the motoneurones are in a state of hyperexcitability to exteroceptive stimuli and that in the case of intradural deafferentation the motoneurones tend to discharge spontaneously

According to Sprong (1929) rigidity is caused by damage to the spinal cord as a result of intradural manipulation because rigidity associated with disturbed bladder function may occur after deafferentation as well as after intentional traumatization of the spinal cord. That an ischemic lesion of the spinal cord produces a rigidity is well known (Gelfan and Tarlow 1959 Biersteker and van Harreveld 1963) In an histological analysis of L7 segments of dogs with ischemic hind limb rigidity Gelfan and Tarlow (1963) found that most of the cells destroyed in L7 segments of three rigid dogs were interneurones and that motoneurones may survive when 80% of the interneurones are destroyed They suggested that the loss of interneurones might account for a state of hypersensitivity of the motoneurones according to Cannon s law (Cannon and Rosenblueth 1949 Stavraky 1961)

In agreement with Ranson (1928) and Sprong (1929) I found that the spinal cord was damaged by the intradural operative procedure Whereas Ranson found bilateral alterations (degeneration in the columns of Clark) in my experiments damage was confined to the side of deafferentation It is difficult to decide what fibre systems have been

affected probably the damage comprises ascending descending and propriospinal fibres in the lateral funiculus The intradurally operated cords showed also a reduced number of neurones on the operated side as compared with the non-deafferented side The absence of muscular atrophy is consistent with preserved motoneurones and damage to interneurones

Therefore it seems justified to assume that the rigidity in intradurally deafferented hind legs of cats is due to damage to the spinal cord The mechanism of how this alpha rigidity (Granit 1955) develops is still obscure hypersensitivity of the motoneurones due to a loss of synaptic connections is a possible explanation (Gelfan and Tarlow 1959)

Although anatomical damage was absent in extradurally deafferented cats (except degeneration of the dorsal funiculus) a certain reduction of synaptic connections to motoneurones is likely to occur This may again result in hypersensitivity of a partially denervated structure and account for the overactivity of extensor muscles

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Effect of Resection of Gastrin Releasing Regions on Acid Response to Sham Feeding and Insulin Hypoglycemia in Pavlov Pouch Dogs

By

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Abstract

OLBE, L. *Effect of resection of gastrin releasing regions on acid response to sham feeding and insulin hypoglycemia in Pavlov pouch dogs.* Acta physiol. scand. 1964. 62. 169—175. — In 9 Pavlov pouch dogs in which antrum-duodenum had previously been excluded from gastrointestinal continuity the antrum was resected. Additional resections of the duodenal bulb and distal 3—4 cm of corpus were performed in 5 of the dogs. Following these resections the acid response to sham feeding was reduced to extremely low values in all animals. The post-resection sham feeding response was markedly increased upon injection of subthreshold amounts of gastrin suggesting that physiological vagal excitation of the HCl glands by sham feeding requires the presence of gastrin to produce significant acid secretion in these preparations. In contrast a substantial post-resection acid response to 0.4—0.6 I.U. of insulin per kg b.w. was still obtained in 6 of 7 dogs. The present results show that the excitations of the HCl glands by sham feeding and relatively high doses of insulin are incommensurable and suggest that vagal activation by these doses of insulin produces acid secretion independent of the gastrin mechanism.

Opinions still differ as to the role of gastrin in the mechanism of HCl gland excitation during gastric acid secretion induced by vagal activation. On the one hand the cephalic phase of gastric acid secretion has been claimed to require the presence of at least part of the antrum (Straaten 1933) and to be controlled by a combined neuro-humoral mechanism in which gastrin from the antrum plays an important role (Uvnäs 1942). On the other hand vagal discharge has been considered to activate the HCl glands directly with gastrin release being a minor and dispensable auxiliary mechanism (Pevsner and Grossman 1955).

The significance of even minute amounts of gastrin in the production of acid secretion by vagal activation was indicated by the fact that subthreshold amounts of gastrin were able to potentiate the effect of vagal excitation of the

HCl glands by sham feeding (Olbe 1964) The purpose of the present study was to investigate the importance of gastrin in the cephalic phase of gastric acid secretion The acid response to vagal activation by sham feeding and insulin hypoglycemia was studied in Pavlov pouch dogs before and after resection of gastrin releasing areas

Methods

Operative Procedures

Nine healthy mongrel dogs were provided with esophageal fistulas (Olbe 1959) and innervated (Pavlov) fundic pouches according to the method of Thomas (1942) At a *second operation the antrum and duodenum were excluded from the gastrointestinal* continuity and a gastrojejunostomy was established At the antrum corpus separation 1—2 cm (dogs B E F G I) or 3—4 cm (dogs M N O) of the acid secreting mucous membrane oral to antrum was excised The distal level of the acid secreting glands was visualized during operation as described by Olbe (1963) and only in dog L were anatomical criteria used for the antrum corpus separation The pyloric ring was resected in all animals and the duodenal bulb was extirpated in dogs E M N O just proximal to the entrance of the common bile duct. The dogs had the antrum and duodenum excluded for a period of 4 to 21 months before the antrum was resected In dogs B and F additional 3 cm of the distal part of the corpus and in dog B the duodenal bulb were later resected

Experimental Procedur s

Before each experiment the dogs fasted for 18—20 hours During the experiments the Pavlov pouch secretions were collected in 15 min period starting with collections from the fasting dogs for at least one hour The amount of acid in the secretions were determined by titration against 0 01 N NaOH with Topfer s reagent and phenolphthalein as indicators In the tables the secretion was expressed in milliequivalents of acid titrated with phenolphthalein as indicator Vagal activation was produced by 10 min sham feeding with minced fish or meat or by i v administration of 0 2—0 6 I U insulin per kg b w Collection of acid secretion proceeded for 3 $^1/_2$ hours with the exception of insulin expts in dog L which proceeded for only 2 $^1/_2$ hours Gastrin was prepared from hog antrum according to the method of Gregory and Tracy (1961) but the purification was stopped after stage one The gastrin preparations had an secretory activity of 42—240 histamine units (Uvnäs and Emås 1961) per mg gastrin The histamine content of the gastrin preparations was *less than 0 1 µg free histamine base per mg gastrin During sham feed*ing expts in dogs B E M N O after resection of the antrum distal part of the corpus and duodenal bulb an i v infusion of a subthreshold dose of gastrin (0 087—0 614 histamine units per kg per min) was performed The subthreshold doses of gastrin used were less than $^1/_3$ of the dose that produced acid secretion in these dogs as determined from a dose response relationship (Table II cf Olbe 1964) The gastrin infusion was started at least one hour before beginning the sham feeding and was continued for further 3 $^1/_2$ hours Each dog received the same gastrin preparation throughout the experimental series

Results

Sham Feeding Response Resection of the antrum in all Pavlov pouch dogs with additional resection of the duodenal bulb in dogs E M N O and the distal 3—4 cm of the corpus in dogs M N O significantly reduced ($p < 0.01$ accord

Table I Mean 3.5 hours sham feeding response in Pavlov pouch dogs before and after resection of antrum distal 3—4 cm of corpus and duodenal bulb and with post resection i v administration of subthreshold amounts of exogenous gastrin (0.296 0.087 0.136 0.160 and 0.614 histamine units/kg/min in dogs B E M N and O cf Table II)

	Mean sham feeding response							
					After resection of antrum distal corpus and duodenal bulb			
	Before resection of antrum		After resection of antrum		Without exogenous gastrin		With subthreshold amounts of exogenous gastrin	
Dog	mEq±SE	No of exp	mEq±SE	No of exp	mEq±SE	No of exp	mEq±SE	No of exp
B	1.05±0.20	4	2.07±0.27	6	0.31±0.10	6	4.31±0.15	2
E	3.26±0.45	5	0.80±0.16	7	0.45±0.03	8	2.03±0.14	4
F	0.76±0.13	4	0.20±0.0[illegible]	3				
G	1.30±0.17	5	0.14±0.04	4				
I	0.49±0.07	5	0.15±0.01	4				
L	0.38±0.07	4	0.04±0.0[illegible]	5				
M	1.37±0.14	4			0.17±0.03	5	1.71±0.27	4
N	1.28±0.[illegible]7	4			0.20±0.04	5	0.8[illegible]±0.15	4
O	0.68±0.14	5			0.21±0.04	5	6.38±0.93	[illegible]

Table II Pavlov pouch responses after resection of antrum (all dogs) distal 3—4 cm of corpus (dogs M N O) and duodenal bulb (dogs E M N O) to intravenous infusion of varying doses of gastrin expressed as the acid output during the 4th 15 min period of the infusion

Dog						
B	did not respond to	1.018 histamine units/kg/min				
E (3 exp)	Dose of gastrin histamine units/kg/min	0.087	0.160	0.494	0.871	1.600
	Acid response mEq±SE	0.01±0	0.03±0.01	0.08±0.04	0.20±0.10	0.20±0.07
M (4 exp)	Dose of gastrin histamine units/kg/min	0.074	0.136	0.420	0.740	1.360
	Acid response mEq±SE	0.01±0	0.01±0	0.07±0.0[illegible]	0.[illegible]0±0.03	0.[illegible]7±0.03
N (4 exp)	Dose of gastrin histamine units/kg/min	0.160	0.419	0.890	1.59	[illegible].814
	Acid response mEq±SE	0.01±0.01	0.05±0.0[illegible]	0.12±0.04	0.19±0.05	0.[illegible]6±0.05
O (1 exp)	Dose of gastrin histamine units/kg/min	0.334	0.[illegible]14	1.897	3.34	
	Acid response mEq	0	0.01	0.0[illegible]	0.24	

Table III Mean 3.5 hours (2.5 hours in dog L) acid response to i.v. injection of insulin in Pavlov pouch dogs before and after resection of antrum, distal 3—4 cm of corpus and duodenal bulb

	Acid response to injection of insulin								
	Before resection of antrum			After resection of antrum			After resection of antrum, distal corpus and duodenal bulb		
Dog	mEq±SE	No. of exp	IU insulin/kg	mEq±SE	No. of exp	IU insulin/kg	mEq±SE	No. of exp	IU insulin/kg
B	0.77±0.07	4	0.2	1.03±0.06	3	0.2	0.15±0.11	4	0.2
							0.65±0.17	4	0.4
E	1.72±0.18	4	0.2				0.26±0.05	4	0.2
							0.77±0.10	4	0.4
G	1.53±0.35	4	0.2	1.68±0.31	3	0.2			
I	0.91±0.31	4	0.2	1.74±0.29	3	0.4			
L	0.22±0.08	4	0.4	0.14±0.06	3	0.4			
M	0.72±0.15	4	0.2				0.12±0.03	4	0.2
							1.31±0.34	4	0.4
N	0.50±0.17	4	0.2				0.10±0.01	4	0.2
							0.38±0.09	4	0.4
							0.74±0.17	5	0.6
O	0.37±0.11	5	0.4				0.16±0.05	4	0.2
							0.51±0.[illegible]2	5	0.4

ing to the Student *t* test) the acid responses to sham feeding in all dogs except B (Table I). The post-resection sham feeding responses were reduced to very low levels with the exception of dogs B and E. In these two cases, however, subsequent resections of the distal 3 cm of the corpus in both dogs plus the duodenal bulb in dog B reduced the sham feeding responses ($p < 0.001$ in dog B and $p < 0.01$ in dog E) to very low levels (Table I). After these operations the remaining acid sham feeding responses of all dogs were so small that no acid determined by titration using Topfer's reagent as an indicator appeared in 28 of 45 expts. No acid appeared in at least 2 expts. of each dog.

Continuous i.v. injection of a subthreshold dose of gastrin during the post-resection sham feeding experiments of 5 dogs (B, E, M, N, O) reestablished substantial acid responses to sham feeding (Table I).

Responses to Insulin Hypoglycemia. After resection of the antrum in all dogs with additional resection of the duodenal bulb and the distal 3—4 cm of the corpus in dogs B, E, M, N, O the acid responses to i.v. injection of insulin in varying dosage were determined. Before resection only one dose of insulin was administered to each dog. The response to 0.2 I.U. of insulin per kg b.w. was substan

tially reduced postoperatively (dogs B E M N) with the exception of dog G (Table III) The postresection responses to this dose of insulin in dogs B E M N O were very small and contained no acid in 14 of 20 expts as determined by titration using Topfer s reagent as indicator No acid appeared in at least 2 expts of each dog However injection of 0 4 (dog B E I L M N O) or 0 6 (dog N) I U of insulin per kg b w resulted in a conspicuous acid response postoperatively with exception of dog L (Table III) In the dogs tested to 0 4 and 0 6 I U of insulin per kg b w the acid responses were significantly higher ($p < 0.001$ according to analysis of variance) than those to 0 2 I U of insulin (dogs B E M N O)

Discussion

Partial gastrectomy has markedly depressed the sham feeding induced gastric acidity in dogs (Straaten 1933) and the acid sham feeding response in human beings (Noring 1951) Cocainization or vagal denervation of an antral pouch has reduced the sham feeding response in Pavlov pouch dogs (Lim and Mozer 1951) supporting the concept that an antral mechanism is involved in the cephalic phase of gastric acid secretion Uvnas (1942) has presented evidence that vagal stimulation of the HCl glands required the concurrent action of gastrin to elicit acid secretion The physiological validity of Uvnas suggestion has been questioned on the basis that he performed acute experiments on anesthetized animals with determination of the acid response to electrical stimulation of the vagi The present study shows that gastrin is required for the production of a significant gastric acid response to physiological vagal activation by sham feeding at least under conditions where antrum duodenal bulb and distal 3—4 cm of corpus have been resected following previous exclusion of the antrum and duodenum from the gastrointestinal continuity

In the present study resection of the gastrointestinal areas known and suspected to release gastrin markedly reduced the gastric acid responses to sham feeding in Pavlov pouch dogs the post resection acid responses to sham feeding being barely discernible with the collection technique used The gastrin releasing areas involve the antrum — defined as the gastric region situated between the distal end of the acid secreting glands and the proximal end of the duodenal mucosa — and possibly part(s) of the mucosa in the immediate neighbourhood of the antrum A gastrin like substance has been extracted from the proximal part of the duodenal mucosa (Komarov 1942 Uvnas 1945) in some species In the present study resection of the distal 3—4 cm of the corpus and or duodenal bulb in addition to the antrum resection proved necessary in 2 dogs to suppress the sham feeding responses to minimal levels The post resection reduction of the acid responses to sham feeding was probably caused by elimination of the gastrin mechanism This conclusion was supported by the finding that following the resections intravenous infusion of subthreshold amounts of gastrin re

established a substantial acid sham feeding response (Table I) The finding suggests that gastrin even in small amounts played a prominent role in the cephalic phase of gastric acid secretion by potentiating the vagal action on the HCl glands The small amount of acid in the post resection sham feeding responses demonstrates the inefficiency of physiological vagal action by itself on the HCl glands in the present preparations

Despite the absence of gastrin releasing areas, vagal activation elicited by injection of relatively high doses of insulin (0 4—0 6 I U per kg b w) produced a definite acid response in 6 of 7 dogs (Table III) all of which produced only a very scanty acid response to sham feeding The acid response to injection of a lower dose of insulin (0 2 I U per kg b w) corresponded better to the sham feeding response being very scanty in 5 of 6 dogs Others (Andersson et al 1958) have failed to reduce regularly the acid response to 0 1—0 2 I U of insulin per kg b w by resection of the antrum in a similar preparation possibly due to incomplete resection of gastrin releasing areas since in these experiments only antrum was resected according to anatomical criteria The present results agree with the findings of Pevsner and Grossman (1955) that vagal activation by injection of 0 5 I U of insulin per kg b w still produced significant acid secretion after resection of the antrum and small intestine suggesting that relatively high doses of insulin (0 4—0 6 I U per kg b w) produces acid secretion without co operation of gastrin In contrast the vagal activation by sham feeding was dependent on the gastrin mechanism to produce significant acid secretion in the present preparations Thus vagal activation by relatively high doses of insulin can give unreliable information about the mechanisms involved in the cephalic phase of gastric acid secretion Hypoglycemia due to these relatively high doses of insulin may possibly induce vagal discharge of an intensity above the physiological range

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Proprioceptive Impulse Patterns during Contraction of Intrinsic Laryngeal Muscles

By

A MÅRTENSSON

Received 19 March 1964

Abstract

MÅRTENSSON A. *Proprioceptive impulse patterns during contraction of intrinsic laryngeal muscles* Acta physiol scand 1964 62 176—194 — In histological serial sections of four different laryngeal muscles in the dog no spindles were found — Evidence of proprioceptive reflexes induced by displacement of laryngeal cartilages and mediated by afferent inflow via the internal laryngeal nerve prompted an analysis of the proprioceptive impulse discharges in this nerve Twitch and tetanic contractions of slow and fast laryngeal muscles were used to induce reproducible movement patterns in the larynx. Comparison with manually applied pressure stimulation indicated that the receptors activated during muscle contraction are located outside the muscles mainly in ligament structures and around joints During twitch contraction receptor activation was observed in the form of an acceleration of spontaneously active units or an initiation of discharges in initially silent units and receptor inhibition in the form of a pause of pre-existent activity The duration of both types of responses could be related to the time course of contraction The same types of effect were induced by tetanic contraction Typical and atypical responses obtained during the dynamic and static phases of tetanic contraction of varying amplitude are described. Most units responded to contractions of two or three muscles, and some common combinations of excitatory and inhibitory effects on one and the same unit are presented It is concluded that the impulse patterns of the receptors studied are differentiated enough to serve a proprioceptive control mechanism

The question whether there are receptors in the dog s intrinsic laryngeal muscles serving a proprioceptive reflex mechanism was taken up for analysis in an earlier paper in which also previous work in this field was reviewed (Martensson 1963) On stimulation of individual muscle nerves and recording from the same nerve or corresponding muscle no monosynaptic reflex responses could be evoked, nor could any afferent impulses be recorded from the nerves to the laryngeal muscles subjected to stretch The results obtained in the functional

analysis were consistent with preliminary data derived from the histological examination of laryngeal muscles in an attempt to establish the presence of spindles These morphological studies have now been followed up and as will be reported in the first section of this paper have not provided any evidence of spindles in the laryngeal muscles of the dog

The laryngeal muscles may however contain other types of stretch receptors the afferent fibers of which do not join the motor fibers but run in the internal laryngeal nerve In the goat s eye muscles proprioceptive afferents and motor fibers have actually been shown to leave the muscle through separate nerve branches (Whitteridge 1955 Cooper and Daniel 1956)

A proprioceptive control may also be exerted by receptors located outside the muscles Andrew (1954 b) made a functional and histological study of the proprioceptors located at the thyroepiglottic joint in the rat and found the behavior of these slowly adapting receptors similar in many respects to that of knee joint receptors (Boyd and Roberts 1953 Andrew 1954 a) From an analysis *of the responses of these proprioceptors to passive movements it was concluded* that they could provide adequate information to the central nervous system about the position and movement of the epiglottis Afferent fibers from joint receptors in the rat s larynx are enclosed in the internal laryngeal nerve which also contains fibers from other types of stretch receptors in the organ the exact locations of which have not yet been established (Andrew 1954 b 1956)

In the course of the present series of experiments on laryngeal functions in the dog stretch applied to the cricothyroid muscle by separation of the cricoid and thyroid cartilages was observed to result in a reflexly evoked increase of its tonic activity although the muscle contains no spindles By nerve section experiments it could be established that the afferent impulses serving this stretch reflex are not mediated through the muscle nerve but via the internal laryngeal nerve From this and from similar experiments it was apparent that a manner of approaching the problem of proprioceptive mechanisms in the dog s laryngeal muscles would be to study the afferent inflow in the internal laryngeal nerve during movements and changes of position of laryngeal structures

In the second section of this paper an account will be given of the results of recordings made from a large number of single units in this nerve representing slowly adapting receptors located outside the muscles mainly in certain ligament structures and around joints The analysis has been limited to impulse patterns set up by movements produced by contraction of individual laryngeal muscles Receptor activation and inhibition occurring during twitch and tetanic contractions will be described and examples given of some common combinations of responses observed in one and the same unit during contraction of different muscles The receptor properties thus observed will be compared with those of previously analyzed proprioceptors The conclusion is drawn that the impulse patterns of the mechanoreceptors studied are differentiated enough to serve a proprioceptive control of the intrinsic laryngeal muscles

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The question whether there are receptors in the dog s intrinsic laryngeal muscles serving a proprioceptive reflex mechanism was taken up for analysis in an earlier paper in which also previous work in this field was reviewed (Mårtensson 1963) On stimulation of individual muscle nerves and recording from the same nerve or corresponding muscle no monosynaptic reflex responses could be evoked, nor could any afferent impulses be recorded from the nerves to the laryngeal muscles subjected to stretch The results obtained in the functional

Results

1 Histological investigation of the intrinsic laryngeal muscles of the dog

Four of the dog s intrinsic laryngeal muscles the thyroarytenoid posterior and lateral cricoarytenoid and cricothyroid were examined histologically In one procedure the whole larynx was dissected out of the animal and fixed for some days in 10 % neutral formalin and then the different muscles with intact attachments were excised by cutting the cartilages The whole muscles were embedded in celloidin (cf Romeis 1948) and consecutive transverse sections cut at 20 μ, every ten sections or about 70—80 per muscle were mounted and stained according to the method of Weigert v Gieson

In another procedure only the thyroarytenoid was excised from its attachments to the cartilages For comparison a length of about 1 cm of the sternohyoid muscle was excised at the level of the larynx After fixation in formalin both muscles were embedded in paraffin and cut serially and transversely at 12 μ Every five sections or about 200 from each muscle were mounted and stained as per above

Microscopical examinations of the sections from the intrinsic laryngeal muscles did not reveal any structures resembling muscle spindles but in the sections made from the sternohyoid muscle estimated to correspond to 10 % of the whole length of the muscle four muscle spindles were identified which exhibited the typical features of such endings such as connective tissue capsule lymph space, nuclear bag and nuclear chain regions (cf Barker 1948)

2 Afferent impulse patterns in the internal laryngeal nerve during contraction of different intrinsic laryngeal muscles

In a series of exploratory experiments it was possible to demonstrate that — although no muscle spindles are present — an increased tonic activity could be induced in the cricothyroid muscle when stretching it by manually separating the thyroid and cricoid cartilages from one another (Fig 1 *A*) That the afferent impulses initiating this "stretch reflex were not transmitted through fibers in the motor nerve was evident since after section of the nerve a reflex discharge could still be recorded from the central end of the nerve when the cartilages were separated This reflex response was however abolished in nerve and muscle after section of the internal laryngeal nerve indicating that this nerve serves as afferent reflex arc In other types of experiment tetanic contraction of one muscle gave rise to a reflex discharge in another muscle as illustrated in Fig 1 *B* however also this reflex discharge was abolished after section of the internal laryngeal nerve

Out of these two possibilities of evoking proprioceptive impulses in the internal laryngeal nerve — muscle contraction or muscle stretch — the first alternative twitch or tetanic contraction has the advantage of evoking reproducible movement patterns which cannot readily be obtained by manual dis-

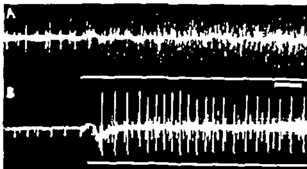


Fig 1 Reflex discharges in cricothyroid muscles *A* increase in tonic activity induced by separating thyroid and cricoid cartilages from one another *B* Sustained discharge induced by tetanic stimulation of contralateral cricothyroid (at 100 per sec) Solid line stimulation period Time bar 200 msec

placement or muscle stretch without a previous operative fixation of the different laryngeal structures It must be taken into account however that even the contraction of an individual muscle implies rather complex changes in the position of the laryngeal cartilages, and these experiments are thus not suited to give information about the position or type of receptor involved By comparison with manually applied pressure stimulation (cf below) it has anyhow been possible to make a rough localization of the receptors showing that they are located outside the muscles and most of them probably in ligament structures and around joints

Seventy units were tested the majority of which were discharging spontaneously *viz* in the absence of any induced mechanical stimulation (cf Andrew 1954 b 1956) Most of them showed steady discharge frequencies varying in different receptors between 15 and 70 impulses per sec In the rest of the fibers cyclic discharges varying with respiration were observed the impulses being as a rule most frequent during the inspiratory but in some units during the expiratory phase Also in receptors initially discharging at a steady rate cyclic discharges were sometimes encountered if respiration became forced in the course of the experiment under such conditions initially silent fibers could in some cases be brought to discharge cyclically

A description will first be given of the afferent impulse patterns obtained during twitch contractions while those induced by tetanic muscle contractions will be described in a following section

Twitch contraction

During maximal muscle twitch two fundamentally different types of effect were obtained *viz* excitatory or inhibitory

Excitatory effects The excitatory effects manifested themselves as an acceleration of the impulse frequency in spontaneously active fibers or as an initiation of discharges in initially silent units Although no strict classification was possible there were great similarities between the responses induced by contraction of the cricothyroid and the posterior cricoarytenoid on one hand and of the thyroarytenoid and the lateral cricoarytenoid muscles on the other hand

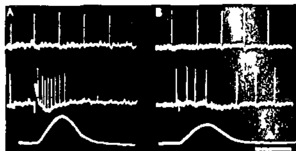


Fig 2 Receptor activation during maximal twitch contractions of slow laryngeal muscles *A* posterior cricoarytenoid *B* cricothyroid (another receptor) From top to bottom resting discharge evoked response contraction curve Time 50 msec

Fig 2 *A* shows a typical afferent response evoked by maximal stimulation of the posterior cricoarytenoid and recorded from a spontaneously active fiber The response consisted of an initial train of impulses followed by a characteristic postexcitatory pause In this case the discharge period corresponded to the rising phase of the contraction curve In other cases the receptor discharges were maintained also during part of the relaxation phase The duration of the discharges varied between 30 and 90 msec while the peak of the contraction curve was attained about 35 msec after stimulation Considerable variations in discharge frequency were observed in different units tested Thus to take an extreme example a response from two different receptors in both cases of a duration of 60 msec might consist of 18 spikes in one case (maximum observed) and of only 3 spikes in the other case The discharges in initially silent units were of fundamentally the same type as those in spontaneously active fibers as appears from Fig 5 *A*

Fig 2 *B* shows a typical response evoked by stimulation of the cricothyroid Also this response consisted of a train of impulses during the rising phase of contraction followed by a silent period which was however less pronounced than in Fig 2 *A* The discharge lasted about 40 msec after stimulation (range 40—70 msec) The repetitive discharges set up by stimulation of the cricothyroid and of the posterior cricoarytenoid are thus of about the same duration as might be expected in view of their similar contraction times (Martensson and Skoglund 1964) The discharge frequency of different units activated by contraction of the cricothyroid varied the average number of impulses in a discharge was generally lower than that found for the posterior cricoarytenoid the maximum observed being 10 during a volley of 60 msec duration In single cases the responses consisted of only one afferent spike thus resembling those obtained by stimulation of the fast laryngeal muscles

During contraction of the faster types of laryngeal muscles the lateral cricoarytenoid and the thyroarytenoid repetitive discharges like those described above were obtained only from a minority of the units tested Fig 3 *A* shows such a response elicited during contraction of the lateral cricoarytenoid The most common type of response in initially silent units during contraction of the

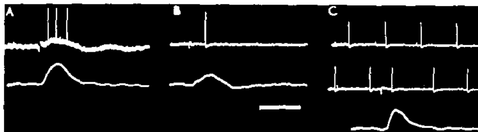


Fig 3 Receptor activation during maximal twitch contractions of fast laryngeal muscles A and B lateral cricoarytenoid initially silent units upper trace evoked response lower trace contraction curve C thyroarytenoid from top to bottom resting discharge evoked response contraction curve Time 50 msec

fast laryngeal muscles consisted of only one spike (Fig 3 *B*) In initially active fibers the responses induced by these muscles appeared as a reduced impulse interval either between the spike immediately preceding and that immediately following upon the stimulus (Fig 3 *C*) or between the two spikes following upon the stimulus The longest latency of such a facilitation was about 25 msec after stimulation The discharge pattern and duration of the responses induced by the two fast laryngeal muscles were similar and corresponded to the contraction courses the peak of the contraction curves occurring in both cases about 20 msec after stimulation (cf Martensson and Skoglund 1964)

As shown in Fig 2 the impulse discharge induced by contraction of the slow muscles was followed by a postexcitatory inhibition The duration of the pause was longer at lower resting discharge frequencies judging from recordings from fibers in which the spontaneous impulse frequency varied cyclically with respiration As shown in Fig 5 *B* the duration of the pause also was longer the larger the number of spikes in the initial impulse volley Silent periods were also occasionally observed after repetitive discharges induced by the lateral cricoarytenoid but in the more common type of response to contraction of the fast laryngeal muscles these pauses were absent or but little pronounced

Inhibitory effects Inhibitory effects of contraction were about half as common as excitatory effects The inhibition was generally recorded as a pause in the discharges in spontaneously active units but could sometimes be observed in initially silent units as a single spike occurring in the relaxation phase of the muscles As in the case of the excitatory effects also the inhibition could be related to the contraction course of the muscles Typical recordings obtained on maximal stimulation of the different muscles are shown in Fig 4 The duration of the pause calculated from the stimulus artefact was 50—60 msec for the responses induced by contraction of the slow muscles (*A B*) and 30—35 msec for those elicited from the fast muscles (*C D*) The duration of the inhibitory effect evoked from the posterior cricoarytenoid varied in different units between 45 and 80 msec and that from the cricothyroid between 35 and 70 msec These variations are in part to be ascribed to fluctuations in the resting frequency the dura

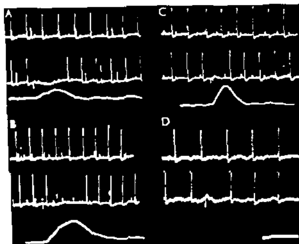


Fig 4 Receptor inhibition during maximal twitch contractions of slow and fast laryngeal muscles A posterior cricoarytenoid B cricothyroid C lateral cricoarytenoid D thyroarytenoid From top to bottom resting discharge evoked response contraction curve Time 50 msec

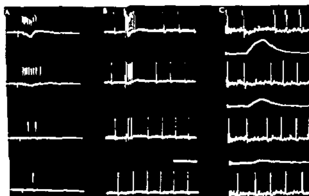


Fig 5 Receptor activation and inhibition during various degrees of twitch contractions A posterior cricoarytenoid initially silent fiber B same muscle spontaneously active fiber C cricothyroid Stimulus strength in all records successively reduced from above downwards Bottom record in B and C resting discharge Time 50 msec in A and C 125 msec in B

tion of the pause being longer the lower the resting frequency the inaccuracy introduced in these measurements by the varying spike intervals is however obvious

Responses to submaximal twitch contractions In a series of experiments the laryngeal muscles were activated by nerve stimuli of varying strengths The variations in the response were most apparent during contraction of the slow laryngeal muscles Fig 5 A shows recordings from an initially silent fiber on stimulation of the posterior cricoarytenoid using successively weaker stimuli As the

stimulus strength was reduced the number of spikes in the volley diminished and the discharge frequency slowed until at threshold only one spike resulted

A similar series of records from a spontaneously discharging unit activated by the same muscle is shown in Fig 5 *B* from which it also appears that the postexcitatory pause is longer the larger the number of spikes in the discharges Responses similar to those described were obtained also during contraction of the cricothyroid muscle

Fig 5 *C* illustrates variations in an inhibitory effect obtained during successively decreasing contraction amplitude of the cricothyroid From a distinct pause during maximal contraction the response changes into a transient retardation in the case of the weakest contraction

Deviations from these typical response patterns were occasionally observed Thus a receptor responding to maximal contraction by an acceleration of the resting discharge followed by a postexcitatory pause might, when subjected to weaker contraction respond only by a pause in the discharge Similar phenomena were observed also during tetanic stimulation as the amplitude was diminished (cf below)

Responses of one and the same unit to twitch contraction of different muscles Most units studied responded to contraction of two or three laryngeal muscles and to judge from the seventy units tested practically all possible combinations of responses may occur the most common of which will be described in some detail below (see also Table I)

The muscles most systematically explored were the posterior cricoarytenoid and the thyroarytenoid which could be activated by both the experimental procedures applied (I and II cf Methods) The most common combination of responses induced by these muscles was receptor activation on contraction of the posterior cricoarytenoid and inhibition on contraction of the thyroarytenoid These types of response were observed in sixteen units Nine of them could also

Table I Some combinations of response types evoked in individual receptors by contraction of different muscles Full explanation in text

	Posterior cricoarytenoid	Thyroarytenoid	Cricothyroid	Lateral cricoarytenoid
A	—	—		
B	-	—	—	
C		—		
D	—			
E	—	-		

+ = receptor activation
— = receptor inhibition



Fig 6 Different types of response evoked in one and the same unit during contraction of different muscles A activation by posterior cricoarytenoid B inhibition by cricothyroid C inhibition by thyroarytenoid Time 50 msec in B and C 125 msec in A

be studied (procedure II) during stimulation of the cricothyroid The result was receptor activation (Table I A) in four and inhibition (B) in five cases Recordings from a unit exhibiting this latter type of response are shown in Fig 6 Of the remaining seven endings which were excited by the posterior cricoarytenoid and inhibited by the thyroarytenoid recordings were made from four during contraction of the lateral cricoarytenoid (procedure I) all four units were activated by this muscle (Table I C)

Inhibition in response to contraction of the posterior cricoarytenoid and activation on stimulation of the thyroarytenoid were observed in eleven units Six of them were also studied during contraction of the cricothyroid and an excitatory effect (D) was induced in four cases One unit was uninfluenced and one inhibited

Eleven units were observed which were activated by contraction both of the posterior cricoarytenoid and the thyroarytenoid Four of these units were tested also on contraction of the cricothyroid and three of them were activated (E) one being uninfluenced

Tetanic contraction

Excitatory effects Most of the units responding to twitch contraction were also studied during tetanic contraction of the muscles Fig 7 A—D illustrates typical responses obtained in a receptor during tetanic muscle stimulation of different strengths In A and B discharges occur only during the rising phase of the contraction curve whereas in C and D discharges are maintained also during the plateau phase As appears from the records the impulse interval is shorter during the rising phase The relation between the discharge frequency in the dynamic and in the static phase of contraction varies in different receptors but this relationship has not been more closely analyzed in this work It should only be noted that a high sensitivity to dynamic stimulation may be observed in some receptors during the plateau phase in the form of a driving phenomenon i e rhythmic discharges occurring in phase with the muscle stimulation in the same manner as previously described for certain muscle endings during vibratory muscle stimulation (Bessou and Laporte 1962 Bianconi and van der Meulen 1963) Such driving phenomena were especially observed during tetanic contraction of the thyroarytenoid in which the tension variations synchronously with the stimuli may amount to 500 dynes at a frequency of 100 per sec (Mårtensson and Skoglund 1964)

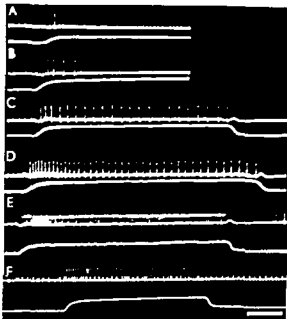


Fig 7 Receptor activation during tetanic contractions *A—D* cricothyroid initially silent unit increasing stimulus strengths maximal in *D* changes in contraction amplitude not proportionally recorded *E* posterior cricoarytenoid and *F* thyroarytenoid spontaneously active units Time 200 msec

When comparing records *C* and *D* it appears that the frequency during the first part of the plateau phase increases with the contraction amplitude in this case it amounts to 40 per sec during maximal stimulation in *D* In *E* is shown a record from another unit during maximal contraction of the posterior cricoarytenoid the discharge frequency is exceptionally high 250 impulses per sec but a relatively high frequency was actually a typical feature in most of the recordings made during contraction of this muscle The effects induced by maximal tetanic contraction may of course vary within a wide range depending on the contractile power of the muscle and the transfer of the mechanical effect to the receptor Thus record *F* shows a receptor activation apparent in the shape of only a moderate acceleration of the resting discharge As appears from all these records there is a slow decrease in frequency during the plateau phase Frequency diagrams illustrating the adaptation rates in two different receptors are given in Fig 11 below

As a rule the excitatory effects evoked by tetanic contraction of the muscles were followed by a postexcitatory pause on cessation of stimulation (Fig 7 *E*) or by a transient slowing-down of the spontaneous impulse frequency below resting level *F* The duration of the postexcitatory inhibition was longer the more long lasting the tetanic contraction and the higher the amplitude of the contraction

Inhibitory effects Fig 8 *A* illustrates a typical inhibitory effect during a maximal tetanic contraction As soon as the stimulus was applied the spontaneous large spike activity ceased and was not resumed until the stimulation was dis

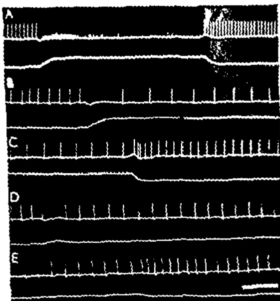


Fig 8 Receptor inhibition during tetanic contraction *A* posterior cricoarytenoid *B* continuing in *C* cricothyroid during maximal contraction *D* continuing in *E* same muscle during submaximal contraction Time 200 msec

continued its frequency was then for a short time slightly above resting level Incidentally it can be seen that during the contraction another initially silent unit of small amplitude becomes activated A similar example of an inhibitory effect during maximal contraction is shown in Fig 8 *B*—*C* In this case the inhibition was complete only during the rising phase of the contraction curve and during the plateau phase the discharge was resumed with successively increasing frequency This behavior is obviously to be ascribed to adaptation processes analogous to those illustrated in Fig 7 During weaker contraction of the same muscle the initial pause as well as the slowing-down of the discharge frequency during the plateau phase were less pronounced (Fig 8 *D*—*E*)

Atypical responses during tetanic contraction In some recordings from units in which an excitatory effect was elicited during twitch contraction tetanic contraction resulted in an accelerated impulse frequency not only during the rising phase of the contraction (Fig 9 *A*) but also in the relaxation phase (*B*

As mentioned above a small number of receptors were found which were inhibited during submaximal and excited during maximal twitch contraction Analogous dual effects were more often observed during tetanic contraction of varying amplitude and the response from such receptors to high amplitude tetanic contraction consisted of an initial inhibition of the resting discharge followed by an acceleration when the contraction curve approached the plateau level (Fig 9 *C*) As appears from Fig 9 *D* the inhibition period can be prolonged by slowly increasing the stimulus from zero to maximal strength thus inducing a very slow increase in contraction amplitude

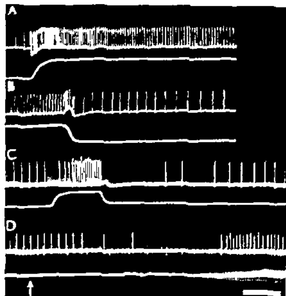


Fig 9 Atypical responses in different receptors during tetanic contractions *A* continuing in *B* posterior cricoarytenoid *C* posterior cricoarytenoid *D* thyroarytenoid Onset of stimulation at arrow Lower beam in *D* muscle action potentials See text. Time 200 m sec

On cessation of stimulation the behavior of the receptors varied depending on their discharge pattern during the contraction phase of the muscle In the cases of receptor responses consisting of a pause or slowing of the impulse rate throughout the whole period of stimulation muscle relaxation may give rise to a rebound effect in the form of an accelerated discharge frequency In cases of receptor responses consisting of an initial silence followed by a discharge of a frequency higher than the resting level of the receptor cessation of stimulation was followed by a postexcitatory pause (Fig 9 *C*) which was of longer duration the more high frequent the previous discharge Brief postexcitatory pauses were also sometimes followed by a discharge during the relaxation phase of the muscle

Responses of one and the same unit during tetanic contraction of different muscles During tetanic as well as during twitch contractions many different combinations of effects were obtained in units responding to stimulation of two or three muscles As a rule the combinations of excitatory and inhibitory effects were identical with those obtained during twitch contraction

Reciprocal responses in one and the same unit during contraction of different muscles can profitably be studied by simultaneous stimulation of two muscles Thus in Fig 10 *A* a sustained discharge has been set up in a receptor by tetanization of the thyroarytenoid During the course of this contraction stimulation is also applied to the posterior cricoarytenoid resulting in an immediate inhibition of the discharge The converse type of reciprocity is shown in Fig 10 *B* A spontaneously active receptor is almost completely inhibited by tetanic contraction of the thyroarytenoid but during periods of activation of the posterior



Fig 10 Reciprocal responses of one and the same unit during simultaneous tetanic stimulation of two different muscles *A* and *B* solid line contraction of thyroarytenoid broken line contraction of posterior cricoarytenoid *C* solid line contraction of cricothyroid broken line contraction of posterior cricoarytenoid See text Time 200 msec

cricoarytenoid the discharge rhythm is resumed and reaches even higher values than the resting discharge Still another example of reciprocity is illustrated in Fig 10 *C* in which a continuous discharge set up in a silent unit by tetanic contraction of the cricothyroid is interrupted by repeated series of tetanic stimulation of the posterior cricoarytenoid

A systematic analysis of the receptor discharges during tetanic contraction is not included in the present study but examples of frequency diagrams from two differently adapting receptors each activated by two muscles are given in Fig 11 One of the receptors showed adaptation curves (*A* and *B*) in which the plateau level was reached within 2—3 seconds after the peak of activation This was a less common finding Usually the adaptation rate was slower as shown by the other example (*C* and *D*) in which the final frequency level was not reached within the 5 second period of stimulation In both receptors cessation of stimulation resulted in pronounced postexcitatory inhibition

Attempts at receptor localizations

Attempts at a rough localization of receptors by mechanically applied pressure stimulation were made in about half the number of units responding to contraction of the laryngeal muscles Judging from the loci exhibiting the lowest stimulus threshold for the initiation of impulses or for changes in discharge frequency these receptors seemed as a rule to be located outside the muscles the most common sites being along the lateral aryepiglottic fold and in structures around the cuneiform and the arytenoid cartilages A small number of receptors seemed to be most sensitive to pressure ventral to the cuneiform cartilage where a connective tissue band links this cartilage to the thyroid cartilage (cf Andrew 1954 b)

In a few cases the lowest threshold for pressure activation of a receptor was found on the belly of the thyroarytenoid muscle Whether this indicates that

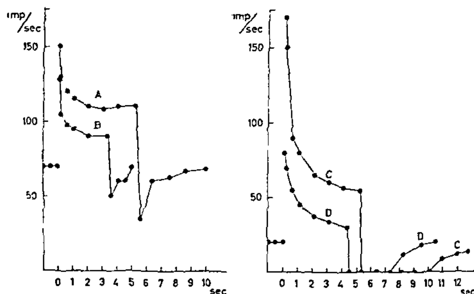


Fig 11 Adaptation of receptor discharge during the plateau phase of tetanic contraction in two different receptors each activated from two different muscles *A* thyroarytenoid *B* lat ral cricoarytenoid *C* posterior cricoarytenoid *D* lateral cricoarytenoid Abscissae time in sec Ordinates discharge frequency See text

some receptors are located in the muscle belly or in the underlying vocal cords cannot be decided In most of these cases an excitatory response was obtained during contraction of the muscle however a pause in a pre existent discharge was never observed which excludes the possibility that the receptor under study was parallel coupled like muscle spindles (Matthews 1933)

It should be stressed however that a more accurate localization of the receptors by means of a manually applied probe is not possible in view of the manner in which the various parts of the larynx are linked together Nor is it possible by manual pressure stimulation to imitate the movements produced by muscular action in order thus to determine the mechanical effect ultimately responsible for the receptor activation Thus *e g* two units in which during contraction of a given muscle opposite (excitatory and inhibitory) effects were recorded were both activated when mechanical pressure was applied at the same site Conversely pressure applied at two points only a few millimetres apart might in one and the same unit induce an excitatory response from one and an inhibitory response from the other of these adjacent loci

Discussion

From the comparative experiments with manually applied pressure stimulation it was evident that most of the receptors studied were located outside the muscles, but it should once more be emphasized that the method used for the

localization of the receptors permits only a rough estimate of their sites Thus for instance, if a receptor was activated by pressure applied to the arytenoid cartilage it could not be decided whether it was located in the capsule of the cricoarytenoid joint in the ligaments or the tendon attachments The fact that in a few cases the lowest threshold for pressure stimulation of a given unit was found on the belly of the thyroarytenoid muscle and that these units were activated during contraction may indicate that special types of mechanoreceptors are present in the muscle (cf *e g* Rudolph 1961)

Of interest in this connection is a recent report by Gracheva (1963) describing various types of nerve endings in the capsule of the cricoarytenoid joint the tendon attachments of some laryngeal muscles and to a lesser extent in their muscle bellies after transection of the superior laryngeal nerve in cat and dog these endings were found to degenerate

Bianconi and Molinari (1962) described experiments on cats in which they studied afferent impulse patterns in single units of the recurrent laryngeal nerve during stretch and contraction of different laryngeal muscles On contraction of the posterior cricoarytenoid and the thyroarytenoid they observed acceleration as well as inhibition of pre existent activity set up by muscle stretch In units inhibited during muscle contraction the discharge frequency could be increased by manually applied pressure to an appropriate area of the muscle and these units were therefore considered to be muscle spindles Although absent in the dog s laryngeal muscles spindles may of course be present in those of the cat (cf Bowden et al 1960) species differences have been demonstrated for instance in the extrinsic eye muscles (cf Cilimbaris 1910 Cooper Daniel and Whitteridge 1951 Cooper and Fillenz 1955)

It should be pointed out however that the responses considered by Bianconi and Molinari to represent muscle spindle discharges were not recorded from separate muscle nerve but from filaments in the main trunk of the recurrent nerve after this had been cut as caudal as possible in the neck According to Kirchner and Wyke (1964) the recurrent nerve of the cat contains afferent fibers from laryngeal joint receptors and the possibility that the afferent responses recorded by Bianconi and Molinari are set up in joint proprioceptors should also be considered In this connection it is noteworthy that attempts to record afferent impulses from muscle spindles in the motor nerve to laryngeal muscles have been unsuccessful both in the rat (Andrew 1956) and the dog (Mårtensson 1963) as also in some exploratory experiments on cat Mårtensson unpublished result)

In the present investigation the discharges recorded from units in the internal laryngeal nerve seemed to originate mainly from receptors located in structures in the cranial part of the larynx No units could be activated by local pressure stimulation of structures in the caudal part such as the cricothyroid joint and the cricothyroid membrane The possibility should be considered that the proprioceptive fibers from the caudal part of the larynx are contained within the

recurrent laryngeal nerve (cf above) or the pararecurrent nerve (cf Lemere 1932) In some exploratory experiments performed in connection with the present series of experiments recordings were made from the latter nerve and impulse discharges similar to those obtained in the internal laryngeal nerve were observed during contraction of different laryngeal muscles A systematic analysis of the various types of afferent responses that can be elicited in units from the recurrent and pararecurrent nerves seems pertinent as a complement to the present study

In previous studies of the cat s knee joint receptors (Boyd and Roberts 1953) responses were recorded from both slowly and rapidly adapting receptors the latter of a type resembling Pacinian corpuscles (Boyd 1954 Skoglund 1956) In the course of the present experiments no responses were obtained from rapidly adapting receptors This does not exclude however that there may be such receptors in structures around the laryngeal joints even though Pacinian like corpuscles have not been histologically demonstrated In the rat rapidly adapting receptors are activated by touch stimulation of the laryngeal mucosa (Andrew 1956) and such receptors in the dog are likely to be stimulated when laryngeal muscles contract their activity would then show up in recordings from filaments of the internal laryngeal nerve However most of the units tested in the present experiments were spontaneously active thus representing a selection of slowly adapting receptors and it is possible that rapidly adapting units would be found if a larger number of initially silent units were tested

It seems to be of interest to compare the properties of the receptors studied in this work with previous data on the slowly adapting type of joint receptors The ability of the receptors under study to maintain discharges from about ten up to several hundred impulses per second is in accordance with previous descriptions of proprioceptors in the knee joint of the cat (Boyd and Roberts 1953 Andrew 1954 a) and in the thyroepiglottic joint of the rat (Andrew 1954 b) As mentioned above the duration of the tetanic contraction used for sustained stimulation of the receptors was limited to maximum 5 sec in order to exclude muscle fatigue and long lasting adaptation processes cannot be studied within these time limits There are however close similarities between the adaptation curves that can be obtained within this period of time (cf Fig 11) and some of the curves published by Boyd and Roberts (1953) relating to knee joint receptors

There is a certain resemblance between the impulse patterns shown in Fig 9 *C* and *D* and the discharges observed in stretch receptors in the knee joint (Andrew 1954 a) and in the thyroepiglottic joint (Andrew 1954 b) During gradually increasing stretch of the medial ligament of the knee as well as during increasing flexion of the epiglottis the initial response of spontaneously active receptors consisted of a pause but as the movement proceeded this response was substituted by an accelerated discharge The explanation offered implied that the receptor passed through a stage of zero stimulation during the

course of the movement The same interpretation may apply to the analogous impulse patterns in Fig 9 *ii2* the receptor may be unloaded during a certain contraction amplitude and then once more be stimulated as the tension increases A similar mechanism may account for the behavior observed in some receptors during the relaxation phase when there is a pause preceding a short burst Whether the type of response illustrated in Fig 9 *A* and *B* consisting of a discharge both at onset and cessation of the tetanic contraction can be explained in terms of mechanical events or is an expression of some specific receptor properties cannot be judged

As mentioned above the different parts of the larynx are intimately connected and contraction of one of the muscles thus gives rise to movements in a number of different parts of the organ Contraction of a given muscle is likely to have differential effects on different receptors depending on their sites The discharge frequency as well as the duration of a receptor response depends — besides on the time course and amplitude of the contraction — on the efficiency of the transmission of the mechanical effect The predominant role of the mechanical forces in determining the response pattern is obvious *e g* from experiments in which one and the same receptor was found to respond to twitch contraction of the posterior cricoarytenoid muscle by a high frequent volley of ten spikes whereas a twitch of the faster and less powerful thyroarytenoid muscle resulted in only one spike However the possible existence of receptors of different sensitivity should also be taken into account

A great number of receptors were found which were reciprocally influenced by the posterior cricoarytenoid and the thyroarytenoid muscles and these endings are obviously situated in structures exposed to mechanical forces of opposite direction when these muscles are activated Since the two muscles act as antagonists in abductor adductor movements in the cricoarytenoid joint a possible location would be in structures around this joint It is of interest in this connection that the capsule of the cricoarytenoid joint of the cat has been shown to have a dense innervation and that the nerve endings embedded in the fibrous capsule are supplied by fibers some of which join the superior laryngeal nerve (Kirchner and Wyke 1964) As appears from Table I receptors were also found which were activated by both the posterior cricoarytenoid and the thyroarytenoid muscles and these must be located at sites where the two muscles exert synergistic effects In view of the complex antagonistic synergistic action of the intrinsic laryngeal muscles it is not surprising that many different combinations of response patterns are represented in the units influenced by different muscles It should be justified to draw the conclusion that the impulse patterns of the mechanoreceptors studied are differentiated enough to serve a proprioceptive control of the intrinsic laryngeal muscles

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Contraction in Intrafusal Muscle Fibres of Xenopus Laevis Following Stimulation of Their Motor Nerves

By

RICHARD S SMITH[1]

Rece ved 31 D cember 1963

Abstract

SMITH R S *Contra t on in int afusal muscle fibres of Xenopus laevi follow n st m lat n of thei moto nerves* Acta physiol scand 1964 6? 195—208 — The contract le beha iou of am phib an ntrafusal muscle fibres following stimula t on of their motor nerves was in estigated by cinephotography of the chang ing sarcomere length Two types of intrafusal muscle fibre were distinguished large nd small Small intrafusal muscle fib es were upplied by small diameter motor nerves and had a lorge lasting contraction than the large intrafusal fibres wh ch were suppl ed by large diameter motor nerves Contract on was a propagated ent in both large and small muscle fib es follow ng single nerve st mul At high st mulat on frequenc es the contract on was not propagated throu h the complete length of th large muscle fib es E idence wa obtained that the large muscle fibres contained, in the region of sensory contacts zones wh ch were less stiff than the resting striated muscle fibre and that the small muscle fibres did not contain uch zones Som observat ons were also mad on spontaneous contract on in these muscle fibres

The results of earlier experiments (Smith 1964) have led to the conclusion that muscle spindles of *Xenopus laevis* contain two types of intrafusal muscle fibre a large diameter muscle fibre having a fast contraction and a small diameter muscle fibre which contracts more slowly These two types of muscle fibre are supplied by large and small diameter motor nerves respectively It was also reported that both types of muscle fibre propagated their electrical impulse across the sensory region of the muscle spindle except on repetitive stimulation when if the stimulus interval were short enough the impulse failed to conduct throughout the entire length of the muscle fibres These conclusions

[1] Med cal Research F llow Med al Research Counc l Canada

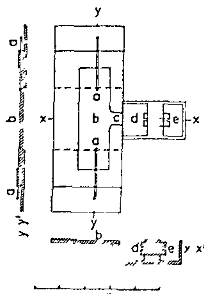


Fig 1 Plan and section views of the recording chamber (a) nylon rods (b) shallow well to contain muscle spindle preparation (c) gap in side of well (d) and (e) pools connected by a glass capillary tube Section X–X passes through (b) (c) (d) (e) and the capillary The broken line beneath the section indicates the position of the condenser during the experiment Section Y–Y passes through the nylon rods (a) and through (b)

are not in complete agreement with other current concepts of the function of intrafusal muscle fibres (Katz 1949 Eyzaguirre 1957 Koketsu and Nishi 1957 *a b*) and since all the previous conclusions have been based largely on electrophysiological evidence it was felt that it was necessary to examine the mechanical behaviour of these muscle fibres in greater detail

Presented here are the results of cinephotography of the behaviour of intrafusal muscle sarcomeres during twitch and tetanic contractions, and of the measurement of the twitch tension in these muscle fibres Some observations on spontaneous contraction of intrafusal muscles and of their behaviour beneath the sensory endings are also described

Methods

Preparation The extensor longus digitorum III IV of the toad *Xenopus laevis* was dissected out with several centimetres of nerve attached The nerve was desheathed from its proximal end to within about one centimetre of the muscle

The dissection of a semi isolated muscle spindle has been described before (Koketsu and Nishi 1957 *a*) but since the experiments reported here do not in all respects confirm the work of others the present technique will be briefly described The muscle was held by its tendons in a chamber similar to that already described (Smith 1961) and the belly of the muscle was supported by a tube of Perspex which stood on the floor of the chamber The muscle was transilluminated and viewed with a dissecting microscope With the muscle fibres aligned it was quite easy to recognise the capsular regions of muscle spindles through the thickness of the muscle Muscle spindles were usually close to the underside of the muscle in association with blood vessels When a suitable muscle spindle had been identified cuts were made with fine scissors across the muscle

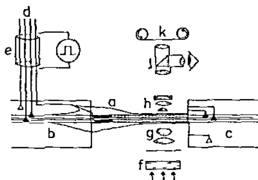


Fig 2 Arrangement shown schematically for photography of intrafusal muscle striations during contraction The dissected portion of the muscle spindle is indicated (a) On the left in an undissected part of the muscle (b) the intrafusal muscle fibres had their motor and sensory innervation intact The extrafusal muscle fibres were all severed but had their motor supply partly intact Intrafusal motor end plates are shown as filled triangles and are arbitrarily indicated Extrafusal motor end plates shown as open triangles The undissected portion of the muscle on the right (c) was completely denervated. The nerve trunk (d) passed through the capillary (e) across which it was stimulated Light from a tungsten lamp passed through a heat filter (f) through the phase contrast condensor (g) the preparation (a) and the phase-contrast objective (h) The prism and lens system (j) allowed the light to pass to the eye or to the film in a 16 mm cine camera (k)

through the extrafusal muscle fibres at a position opposite the spindle capsule After the majority of the extrafusal muscle fibres had been severed the rest of the muscle fibres surrounding the muscle spindle were cut through either with steel needles or tungsten wires which had been electropolished to a fine point Extrafusal tissues were dissected away from the spindle capsule by cutting through connective tissue strands by pressing them against the Perspex support with dissecting needles

The preparation was always immersed in Ringer s solution with the composition (mM) NaCl 112 0 KCl 2 5 $CaCl_2$ 2 0 $NaHCO_3$ 2 5 and with a pH of 7 5 to 8 0

Measurement of sarcomere length The nerve muscle preparation was transferred from the dissecting chamber to a recording chamber without stretching and without taking it through a saline air interface The recording chamber which was constructed for use on a microscope stage is shown in Fig 1 The muscle was held by its tendons between two split nylon rods (a) in a 0 5 mm deep well (b) while the nerve was drawn out through a gap in the side of the well (c) and through a capillary between the pools (d) and (e) The capillary provided an external high resistance across which the nerve could be stimulated at threshold to activate large motor fibres and supramaximally with a distally applied anode block to obtain selective small motor fibre stimulation (Smith 1964) Silicone grease or petroleum jelly was smeared on the top of the walls of the shallow well containing the muscle and a cover slip prevented evaporation of water from the solution bathing the muscle and gave a plane optical surface to the top of the Ringer s solution in the well

The complete experimental arrangement for photography of the intrafusal muscle sarcomeres is shown schematically in Fig 2 The diagram illustrates that (1) the intrafusal muscles were photographed at a position some distance past the termination of the sensory endings and at the end of the spindle capsule distant from the innervated portion of the muscle (2) the muscle fibres were photographed through the spindle capsule this often led to poor optical conditions (3) both intrafusal muscles and extrafusal muscles were innervated on the side of the dissection close to the entry of the nerve trunk but the extrafusal muscles were all severed (4) the intrafusal muscles and extrafusal muscles were denervated on the side of the dissection far from the entry of the nerve trunk

The chamber described above and shown in Fig 1 fitted onto the stage of the ami scope with phase contrast optics Ag AgCl electrodes were also attached to micro orc

scope stage and could be lowered across the capillary containing the nerve Stimulation current was supplied by a rectangular pulse generator

Light from a low voltage tungsten lamp passed through a collecting lens to give Kohler illumination and through a bath of 10 % $CuSO_4$ solution which acted as a heat filter A 3 6 mm Reichert phase contrast objective with a numerical aperture of 0 65 and a working distance of 0 52 mm was used to form an image of the preparation which could be viewed by means of a prism by eye or when the prism was rotated out of the optical path by a 16 mm cine camera

The camera was supported by a heavy stand and did not touch the microscope The film transporting mechanism was calibrated photoelectrically and gave a stable film speed of 37 frames/sec. Movement of the film was at right angles to the long axis of the muscle fibres While the preparation was being viewed by eye a dark green filter was interposed between the light source and the heat filter The green filter was removed during photography The temperature of the fluid in the muscle chamber was checked with a thermocouple and was found to remain at room temperature which in this series of experiments varied between 20 and 23 C

Ilford FP3 16 mm film was used and the development time was prolonged to give an emulsion speed greater than the manufacturers rating

To measure the average sarcomere length the negatives were projected onto a ground glass screen and the total length of the same ten sarcomeres was measured on successive frames with vernier calipers In some cases large prints were made from the negatives and measurements were taken from these

Tension measurements To measure twitch tension developed by the intrafusal muscle fibres the preparation was mounted in a chamber similar to that described by SMITH (1964) except that in this case the muscle was vertical The lower tendon was attached to the floor of the chamber and the upper tendon to a freely pivoted lever which rested on the anode pin of an RCA 5734 mechano-electric transducer The resonant frequency of the lever and the anode pin together was 2 kc In order to avoid artifacts produced by vibrations transmitted from the floor of the building a massive elastic mounting was used to support the apparatus The output of the transducer was amplified with a D C amplifier and displayed on an oscilloscope In order to view the muscle and muscle spindle while tension was being recorded a dissecting microscope was mounted horizontally and the preparation was viewed through the Perspex wall of the chamber During the experiments it was found that when the muscle was stretched and mounted then the extrafusal muscles which were innervated did supply some measurable tension on stimulation of the nerve even though all the muscle fibres were cut To avoid artifacts of this sort the extrafusal end plates were blocked with 3 × 10^{-6} g ml d Tubocurarine This curarization did not affect the activity of the intrafusal muscle fibres At the end of the experiment the dissected portion of the muscle spindle was squeezed and a check was made to determine whether the disappearance of movement in the intrafusal muscle fibres coincided with a disappearance of twitch tension

Results

Direction of impulse movement Following a stimulus to the nerve the direction of movement of the contraction wave in the intrafusal muscle fibres could easily be seen when the preparation was viewed at low magnification The wave moved always from the end of the preparation at which the nerve trunk entered the muscle towards that end farthest from the entry of the nerve trunk (see

Fig 2) This observation was taken as evidence that that part of the muscle spindle which was farthest from the entry of the nerve trunk had in fact been denervated by the dissection

Region of sensory contact It was noticed that in the region of sensory contacts at least some of the muscle fibres contained a zone in which no striation could be seen even at high magnification In several cases the length of this zone could be measured and was 70—90 μ long Stimulation of the nerve with a threshold stimulus revealed that this zone was contained in the large (Smith 1964) muscle fibres The apparently striation free zone had mechanical properties which were obviously different from the rest of the muscle fibre This zone clearly extended when the rest of the muscle fibre contracted The sequence of events observed following stimulation of the nerve and during the subsequent contraction of the muscle fibre was that the striation free zone extended first towards the innervated end of the muscle fibre then also toward the denervated end of the fibre Finally the whole zone relaxed symmetrically to its original length In other words the striation free zone acted as though it were an elastic structure with a stiffness less than that of the striated part of the muscle fibre in the resting state

A striation free region in intrafusal muscle fibres has long been known in frog muscle spindles and has been described recently by Gray (1956) in fixed preparations Jahn (1959) described a zone of about 100 μ length in which no striation could be seen in living muscle spindles A region the reticular zone has been described by Katz (1961) which measured from 50—100 μ in length and which contained few myofibrils The extensible region seen in the present experiments may well correspond to the reticular zone

An extensible striation free region was not seen in the small intrafusal muscle fibres which contract alone when the nerve to the preparation is stimulated supramaximally with an anodal block applied to large nerve fibres Although this does not exclude the presence of such a zone in the small muscle fibres it should be mentioned that Jahn (1959) observed that some muscle fibres could traverse the region of sensory contacts without losing their striation at any point

Spontaneous contractions Spontaneous contractions in frog intrafusal muscle fibres have been reported by Buchthal and Jahn (1957) In the present investigation of the properties of amphibian intrafusal muscles over two hundred muscle spindles have been observed and spontaneous contraction of the intrafusal muscle fibres was seen in approximately 10 of the preparations It was noticed that spontaneous contraction could be associated with an ageing preparation but that this was not always the case

The phenomenon was briefly investigated in the following way

a The muscle was examined under the dissecting microscope while *in situ* with the circulation intact Transillumination of the muscle was achieved by leading light beneath the muscle with a 1 mm thick strip of Perspex Even

though blood corpuscles could easily be seen no muscular movement was observed

b No spontaneous contraction of intrafusal muscle fibres was seen in muscle spindles observed through the intact mu cle *in vitro*

c Spontaneous contraction of intrafusal muscle fibres was rarely seen (see above) in dissected preparations even though the muscle fibres would contract in response to a ingle nerve stimulus

d At short times after the end of fast repetitive stimulation of the nerve one or more spontaneous contractions of the intrafusal muscle fibres could be seen

e The effect of modifying the calcium and potassium concentrations in the Ringer s solution on the behaviour of intrafusal muscles was studied in undissected and dissected muscle spindles *in vitro* Lowering the [$CaCl_2$] from 2 0 mM to 0 5 mM caused spontaneous contraction of the intrafusal muscle fibres In whole muscles these contractions took place in the complete absence of any extrafusal muscle contraction The contractions which started almost immediately on changing solutions occurred initially at a very high rate and settled down after a minute or two to a steady rate of about 1—0 5/sec Similar spontaneous contractions were seen when the [KCl] was raised from 2 5 mM to 5 mM Both these effects were completely reversible and neither was abolished by the addition of d Tubocurarine in a concentration of 1 5 × 10^{-6} g/ml

It was concluded that pontaneous contraction was not normal in the muscle spindles investigated However it was apparent that spontaneous contractions of intrafusal muscle fibres can occur under various circumstances

There are several peculiarities of the excitability of intrafusal muscle fibres that may be related to the phenomenon of spontaneous contraction as described by BUCHTHAL and Jahn (1957 Differential blocking of neuromuscular transmission between intra fusal and extrafusal muscle fibres by critical curarization has been described in Amphibia (Katz 1949 Eyzaguirre 1957 Henatsch and Schulte 1958 Smith 1964 On the basis of his observations on critically curarized muscles Katz (1949) suggested that there is a high safety factor for neuromuscular transmission in muscle spindles and suggested that this may depend on the relative sizes of the nerve ending and the muscle fibre. This is no doubt a contributing factor to a high safety factor however the threshold of intrafusal muscle fibres could be lower than that of the extrafusal muscles in fact there is evidence to indicat that this second possibility may be true

The effect of calcium on nerve cell membranes has been analysed in detail (Frankenhaeuser 1957 Frankenhaeuser and Hodgkin 1957 While calcium has complex effects on the permeability mechanism of cell membranes it can be said that moderate decreases in external calcium concentration decrease the depolarization required to reach threshold Thus while other explanations might well hold it can be said that intrafusal muscle contraction in the absence of extrafusal muscle contractions in low calcium could indicate a lower initial threshold in the intrafusal muscle fibres This effect is certainly on the muscle membrane itself since it is not altered by d Tubocurarine Raising the external potassium concentration will depolarize both intra and extrafusal muscle fibres with the concentration changes used here the depolarization might have been

Table I Diameters of intrafusal muscle fibres having 'fast' and 'slow' twitches as seen by eye through the phase contrast microscope The fast movement was always associated with threshold stimulation of the nerve The slow twitches were produced by selectively stimulating small diameter motor axons Only those preparations in which the diameters of all muscle fibres could be measured and in which all muscle fibres had intact motor innervation are included

Preparation	Diameters fibres with fast movement		Diameters fibres with slow movement
1	16 μ	15 μ	11 μ
2	14 μ	14 μ	10 μ
3	16 μ	13 μ	10 μ
4		15 μ	7 μ
5	13 μ	9 μ	8 μ

about 15 mV (Hodgkin and Horowitz 1959) Intrafusal muscle fibres began to contract when depolarized in this way while the extrafusal muscles did not This observation could also be explained if the intrafusal muscles had a low threshold

Spontaneous contractions as observed by Buchthal and Jahn (1957) could occur if the threshold of the intrafusal muscles were close enough to the resting membrane potential and if the membrane potential were being perturbed by random liberation of transmitter substance from the extensive motor end plate regions Indeed muscle spindles which otherwise showed no spontaneous contractions did in the present investigation contract spontaneously following the cessation of a repetitive stimulus to their motor nerves It has been shown that in mammalian muscle the frequency of random liberation of transmitter substance is greatly increased following repetitive stimulation of the motor nerve (Brooks 1956 Liley 1956 *a* *b* Hubbard 1959)

Muscle diameter and speed of movement It was reported earlier (Smith 1964) that the movement of the intrafusal muscle bundle in response to stimulation of small motor nerves was clearly much slower than the movement due to stimulation of large motor fibres Other evidence enabled the conclusion to be drawn that the slow movement was property of a small diameter muscle fibre Microscopic examination of paraffin sections and of teased fixed whole spindles suggested the presence of two groups of intrafusal muscle fibres with diameters of approximately 15 μ and 7 μ (Smith 1963) In the present investigation the movements as seen by eye through the phase contrast microscope of individual intrafusal muscle fibres were classified as slow and fast The results are presented in Table I Only those preparations in which it was possible to measure the diameter of all the muscle fibres and in which all the muscle fibres contracted following stimulation of the nerve trunk are included in the table The diameters of the muscle fibres were measured to the nearest micron at a point in the polar region of the muscle spindle In each case the smallest muscle fibre was found to be the slowly contracting one The difference between the diameters of the smallest fibre in the spindle and the smallest fibre having

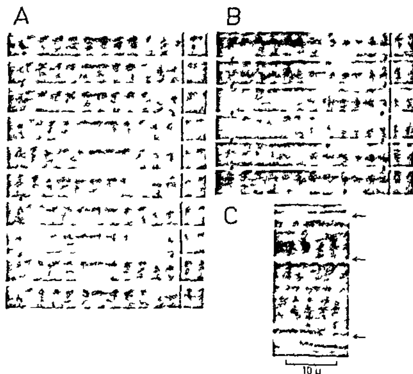


Fig 3 Portions of enlarged prints made on a high contrast paper from 16 mm negatives arranged to illustrate sarcomere shortening during contraction of small and large intrafusal muscle fibres. A. Small intrafusal muscle fibre. Selective stimulation of small motor nerves. Strips cut from enlarged prints of successive frames of 16 mm film taken at a speed of 37 frames/sec. Striations at the left of the strips are aligned as indicated by dots. Position of one and the same striation on the right of the strips also indicated by dots while the vertical straight lines are intended only as a guide for the eye. The striation indicated by dots on the right hand side of the strips shows a movement towards the left hand side of the illustration. B. Large muscle fibre. Selective stimulation of large motor nerves. Procedure and marking of strips as explained above. Sarcomere length of each muscle fibre during contraction is plotted in Fig 4. C. Part of a 16 mm negative enlarged to show muscle fibre diameters. Small muscle fibre approximately 7 μ in diameter. Large muscle fibre approximately 15 μ in diameter. Temperature 22° C. Fibres included in Table I, preparation 4.

a fast movement may not have been marked (e.g. Table I, preparation 5) but no particular significance is attached to this observation since it was sometimes observed that the fibre diameters and their differences became greater at greater distances from the sensory region. Barker and Cope (1962) have reported that frog intrafusal muscle fibres increase in diameter away from the sensory region.

Measurement of sarcomere length. The main object of the experiments reported here was, as stated before, to measure the time course of the change in sarcomere length in intrafusal muscle fibres during stimulation of their motor nerves. The foregoing general observations serve largely as an introduction to the fol-

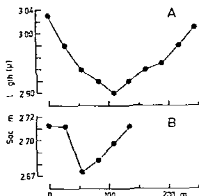


Fig 4 The average sarcomere length of the muscle fibres illustrated in Fig 3 plotted during contraction Each measurement of sarcomere length was obtained from on of the strips of Fig 3 Temp rature 22 C (A) Change in sarcomere length during contraction of small muscle fibre Nerve to p eparat on stimulated selectı ely for small nerve fibres (B) Change in sarcome e length during contract on of large muscle fibre Stimulation of nerve to preparation at threshold Fibres included in Table I p eparation 4

lowing measurements and also indicate the functional state of the muscle fibres in these preparations

The position of the sarcomeres at equally spaced intervals of time during contraction of the fibres was recorded on film as described and the length of usually ten sarcomere units was measured The same set of sarcomeres was used for each measurement as illustrated in Fig 3 For the purpose of graphical presentation the measurements were reduced to length per sarcomere

The length of the muscle spindle was adjusted so that the same set of sarcomeres remained within the photographic field during a contraction the only exception to this being those experiments involving tetanic contraction of the muscle fibres Small movements of the preparation were usually obtained by extending the muscle spindle just only until the intrafusal muscle fibres were straight Since the width of the photographic field was only about 50 μ longitudinal displacement of the preparation had to be limited to a few microns If this precaution were not taken photography of single twitches became impossible since the viewed part of the preparation moved rapidly acro s and out of the field and usually moved out of focus at the same time

The time courses of the change in sarcomere length during single twitches of the individual muscle fibres in preparations 4 and 5 of Table I are illustrated in Fig 3 4 5A 6A 7A In Fig 3 the striations on the left hand side of the illustrations are aligned as indicated by the points thus producing a relative movement of the sarcomeres at the right hand side of the illustration during the contraction It is plain that the duration of the measurable change in sarcomere length is greater in the small muscle fibres which responded to stimulation of small motor nerves than in the large fibre which responded to stimulation of large motor nerves The difference shown by these measurements however would hardly seem to be sufficient to enable the contractions to be classified fast and slow by mere visual observation

Smith (1964) reported that on repetitive stimulation the electrical impul e failed to pa s through the sen ory region of the spindle in both large and small

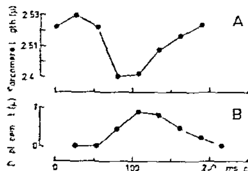


Fig 5 (A) Averag sarcomere length during contraction of large intrafusal muscle fibre. Stimulation of large diameter motor nerves (B) Displacement of sarcomeres in longitudinal axis of the fibre during contraction The abscissae of (A) and (B) represent *the same* period of time Temperature 22 5 C. Fibre diameter 13 μ Fibre included in Table I, preparation ɔ

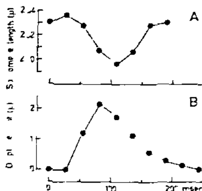


Fig 6 (A) Average sarcomere length during contract on of large intrafusal muscl fibre Stimulation of larg diameter motor nerves. (B) Displacement of sarcomeres in longitudinal axis of the fibre during contraction. The abscissae of (A) and (B) represent *the same* period of time Temperature 22 ɔ C. Fibre diameter 9 μ Fibre included in Tabl I preparation 5

muscle fibres Attempts were made in the present experiments to detect the mechanical result of impul e blockage which should be an increase in sarcomere length at sites past the block The criterion for a successful experiment was that the sarcomere length should become greater than its resting length when the fibre was stimulated repetitively This effect was not detected in the small muscle fibres The effect was however observed in the large muscle fibres in several experiments In one experiment the sarcomere length increased from 2 ɔ to 2 6 μ when the fibre was stimulated via its nerve at 50 sec These experiments were technically difficult and while it is regarded as significant that this effect was detected in the large muscle fibres not much significance is attached to the failure to detect the effect in the small muscle fibres

Intrafusal muscle shortening and tension development Eyzaguirre (1957) noted that the intrafusal bundles seemed to horten freely when the muscle spindles were stimulated in whole muscles The muscle spindles in the preparations used here behaved in the same way this is pos ibly due to the fact that the intrafusal muscle fibres run for the greatest part of their course inside a connective tissue tube this arrangement probably places them more effectively in parallel with the extrafusal fibres which are to some extent bound together by

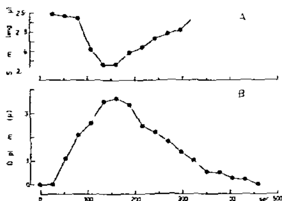


Fig 7 (A) Average sarcomere length during contraction of small intrafusal muscle fibre Stimulation of small diameter motor nerves (B) Displacement of sarcomeres in longitudinal axis of fibre during contraction The abscissae of (A) and (B) represent *the same* period of time Temperature 22 5 C Fibre diameter 8 μ Fibre included in Table I preparation 5

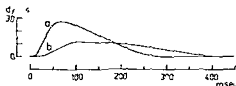


Fig 8 Tracings of tension records obtained following stimulation of either large diameter motor nerve fibres (a) or small diameter motor nerve fibres (b) Each tracing begins at time zero with the delivery of the stimulus to the nerve Temperature 21 C

connective tissue In this case the movement of intrafusal muscle fibres can be regarded as a semi isotonic shortening the load being applied to the ends of the fibres via elastic connective tissue In these experiments the muscle spindle was arranged so that the length of the whole preparation was just sufficient to keep the intrafusal muscle fibres straight thus the muscle fibres were lightly loaded Under these conditions the shortening of the muscle fibres caused a displacement of the sarcomeres in the longitudinal axis of the fibre To clarify this point it should be mentioned that the spindle capsule usually selected for dissection was that lying most distally in the muscle hence the intrafusal muscles were observed at a point close to their distal ends and the displacement was always towards the proximal innervated end of the fibre The movements as seen by eye of the individual muscle fibres which led to the description of fast and slow movements in Table I were thus a combination of shortening of the sarcomere length at the point of observation and of a displacement of the sarcomeres in the longitudial axis of the fibre due to shortening of the whole muscle fibre The displacement of the sarcomeres during the contractions illustrated in Fig 5A 6A 7A are shown in Fig 5B 6B 7B

Tracings of tension records obtained as described under Methods on streched muscles in which the conditions presumably were approximately isometric are shown in Fig 8 This particular pair of tracings was selected since it represents the least difference between the peak tensions developed on stimulating the large and the small motor nerve fibres and so probably represents the contractions of single large and small intrafusal fibres

It is surprising that the large muscle fibres which deliver a greater peak tension also show a shortening or displacement as measured above which is less than that shown by the small muscle fibres This observation could be explained if the small muscle fibres were much longer than the large muscle fibres on the other hand there is no evidence that this is the case and in fact there is evidence to the contrary (Barker and Cope 1962) The load applied to the ends of the fibres could also be quite different and no doubt differing conduction velocities in the two types of muscle fibres would also contribute towards the effect It has already been pointed out however that extensible non striated zones were seen in the large muscle fibres and that these regions behaved as though they were less stiff than the striated portion of the muscle even in the resting state If such zones which have a low stiffness are inserted at intervals in the large muscle fibres then the total shortening of the muscle fibre during contraction will not be as great as in a normal fibre under the same loading conditions The argument can also be used to suggest that the small intrafusal muscle fibres contain no such extensible zone

These conclusions could be tested although the test might be complicated by the possible unequal length of the intrafusal muscle fibres by observing the increase in sarcomere length in the two types of muscle fibre on passive extension of the spindle

The main conclusions reached from the evidence presented in this report are that large intrafusal muscles were supplied by large motor nerves and had a shorter lasting contraction than the small intrafusal muscle fibres which were supplied by small diameter motor nerves The propagated impulse elicited a contraction on both sides of the sensory region in both large and small muscle fibres following single nerve stimuli but at high stimulation frequencies contraction was limited in the large muscle fibres to the innervated end of the muscle spindle There is evidence that the large muscle fibres contained in the area of sensory contacts regions which were much less stiff than the resting striated muscle and this region extended during the contraction of the rest of the fibre The evidence was further that the small muscle fibres did not contain similar regions

Discussion

The work reported here has confirmed in several important respects the conclusions drawn from earlier experiments (Smith 1964) Intrafusal muscle fibres having the smallest diameter were found to have a longer lasting contraction than the larger intrafusal muscle fibres The small muscle fibres were supplied by motor nerves having a diameter smaller than the nerves which supplied the large muscle fibres Sarcomere shortening could be measured in both types of muscle fibre at a point on that side of the capsular region distant from the motor innervation and the contraction wave could actually be seen to travel from the

innervated to the denervated side of the muscle spindle From these observations it was concluded that a propagated impulse elicited a contraction along the complete length of both types of muscle fibre It was also shown that at high rates of stimulation the contraction in the large intrafusal fibres could become localized These conclusions had also been reached on the basis of the earlier work The objection could be raised that the small muscle fibres might be supplied by motor nerves having an end plate that traverses the capsular region of the muscle spindle and it is true that these experiments did not distinguish between conduction in the muscle cell membrane itself and conduction in another structure outside the muscle cell However since motor end plates which traverse the sensory region have not been observed either by light microscopy (Gray 1956) or by electronmicroscopy (Katz 1961) it must be concluded that the small intrafusal muscle fibres do not have the properties described for extrafusal slow muscle fibres (Kuffler and Vaughan Williams 1953 *a b*)

Another feature of amphibian intrafusal muscle fibres has been suggested by the evidence presented here and by earlier electrophysiological evidence (Smith 1964) The electrical evidence suggested that the large intrafusal muscle fibres contain in the region of the sensory endings a zone of low safety factor for conduction of the electrical impulse The available evidence was that the small intrafusal muscle fibres contain no such zone in a comparable location On the basis of these observations it was suggested that the zone with the low safety factor could be the reticular zone described by Katz (1961) Katz described the reticular zone as being from 50—100 μ in length the myofibril content of the muscle fibre is low within this zone Katz did not observe such a zone in the smallest intrafusal muscle fibres The results of the experiments reported here have also suggested a difference in structure between the two types of intrafusal muscle fibres namely that the large muscle fibres contain beneath the sensory endings a non striated zone approximately 100 μ in length with a stiffness less than that of the resting striated fibre and that the small muscle fibres contain no such zone The differences between the two types of muscle fibres allow the speculation that they may have quite different roles in the control of the sensory discharge from the spindle The extent to which an analogy can be drawn between amphibian and mammalian intrafusal muscle fibres is uncertain That mammalian nuclear bag and nuclear chain intrafusal muscle fibres perform different roles in controlling sensory discharge has been strongly implied by the work of Jansen and Matthews 1961 1962 Appelberg (1962 and Granit and van der Meulen 1962 There has been considerable speculation concerning the mechanical properties of intrafusal muscle fibres and Jansen and Matthews 1961 1962 have selected one possible arrangement in order to form the hypothesis that the nuclear bag fibres mostly affect the phasic discharge of the sensory endings while the nuclear chain fibres have their main effect on the static sensory discharge

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Acta physiol scand 1964 62 209—217

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Studies on Heart Lymph

I Kinetics of ¹³¹I Albumin in Dog Heart Lung Preparations

By

N H Areskog G Arturson and G Grotte

Received 4 February 1964

Abstract

Areskog N H G Arturson and G Grotte *Stud es on heart lymph I Kinetics f I-albumin n d g heart lun pr paratio s Acta physiol scand 1964 62 209—217* — The transport of I albumin from plasma to lymph was stud ed in dog heart lung prepa ations Cannulation of a lymph vessel mainly draining the left v ntricle was performed After injection of the labelled substance plasma mixing was complete w thin a few minutes The plasma concentrat on of the tracer was constant for 2—3 hours During this time plasma and heart lymph samples we e collected The increase in the concentration of the labelled substance i heart lymph showed a time course indicating that the substance from the intra vascular compartment was mainly distri buted to a s ngle extra vascular compartment. The data could thus be fully explained by a two-compartm nt model a d the permeab lity constants and the s e of th e tra ascular albu m n sp ce drained by the lymphat c cannula could be calculated.

The present experim ental approach th efore allo ed a quant tative lymph experiment i e. the size of the extra vascular albumin space drained by a lymphatic can ula could be defined and also an idea of the amount of albumin filtered ac oss the cap llary walls and the rate of emoval of albumin from the extra vascular space via the lymphatics was obta ned

Drinker and Field stated in 1931 that blood capillaries universally leak protein and that this protein is returned to the circulating blood via the lymphatics The extra vascular circulation of protein has since then been confirmed by many workers (cf reviews by Drinker and Yoffey 1941 Yoffey and Courtice 1956 Mayerson 1963) Using plasma proteins labelled with a rad o-active isotope or ith T1824 the passage of proteins from plasma to lymph in various regions has been investigated and these earlier investigations have shown that plasma proteins slowly escape across the ca pillary walls m x with another extra vascular protein pool and are returned to the blood via the l mphatics Prot in leakage seems to vary in different tissues, as estimated by the varying protein concentrations in lymph from different regions of the body

These differences in protein concentration may be due to a number of factors such as differences in permeability capillary pressure lymph flow re absorption of water etc

It is difficult to estimate the precise magnitude of the extra vascular protein pool and its turnover The size of the total extra vascular protein pool has been estimated to be of the same order of magnitude as that of the intra vascular It has however not been possible in lymph experiments to calculate with any degree of certainty the magnitude of the extra vascular pool in any given region of the body or of any special organ

From plasma disappearance of labelled protein and measurements of transport of protein in various lymphatic ducts it seems reasonable to assume that at least 50 % of the circulating proteins pass through the capillary walls to the extra vascular com partmen and back each day (cf Yoffey and Courtice 1956 Wasserman *et al* 1956) The dynamic equlibrium of the plasma proteins of the mammal has been further analyzed and discussed by Berson *et al* (1953) Gitlin (1957) and Gitlin and Janeway (1960)

Numerous studies have been performed in which the rate at which labelled proteins pass from plasma to lymph has been determined The system has however usually been too complicated for a more detailed analysis of the data and the information obtained has usually been limited to steady state concentration ratios appearance times and the amount of protein transported via the lymphatic ducts

In the present study a quantitative method of determining the kinetics of albumin in the dog heart lung preparation is described

Methods

Ob rati e t chnique

Eight young mongrel dogs weighing 15– 25 kg and of both sexes were used Nembutal® anesthesia (Veterinary Nembutal® Abbott 60 mg/ml) was used in all experiments For induction of the anesthesia 30 mg/kg b w was given intravenously

Can ulation of the heart lymphatics

Transverse incision over mid sternum or vertical section of the sternum in the mid l ne was used both techniques given good exposure of the base of the heart As soon as the chest was opened artificial respiration was instituted by means of a respirator The lymph gland or glands situated between the superior vena cava and the brach ocephalic artery were identified about 2 cm above the base of the heart as described by Drinker *et al* (1940) A mass ligature was tied around the soft tissues above these glands In about 90 of the cases it was then possible to identify a larger lymphatic running along a fine nerve structure from the bas of the heart somewhat obliquely up to the lymph gl nds mentioned above Often th s lymphatic divides into two or more trunks before entering the lymph gland This main trunk was then cannulated us ng a PE 0 polyethylene cannula After each experiment Patent Blue dye was injected into the wall of the left ventricle of the he rt to check that the cannulated lymphatic trunk was actually draining the l ft ventricle and to determine the appearance time

The Starling hea t lung p epa ation (HLP)

A mod fied dog heart lung preparation was used The brachiocephalic trunk was cannulated on the arterial side and the superior vena cava on the venous s de Before the cannul tion all small vessels pas ing from the pericardium to the med astinum and thymus were ligated to get the HLP free from leaking blood The resistances on the art rial and venous s de were kept constant throughout th experiment The arter al cannula was connected to a b g Wind kessel to simulate the volume elastic ty of the larger arteries Arterial and right atrial pressures and cardiac output minus coronary blood flow were re orde l continuously Heart rate and temperature were recorded at frequent intervals A more detailed description of the HLP method has been publ shed elsewl ere (Areskog 196?)

The test substance

Fresh human or dog ¹³¹I albumin with an initial specific activity of about 5—10 μC per mg albumin was used. Free iodine was less than 2 The total doses given to each dog were about 150μC. The radio-activity was detected by means of a well type scintillation counter and sufficient counts were accumulated to have not more than 5 standard deviation (S.D.)

The protein determination

The total serum and lymph nitrogen was measured by the Kjeldahl method and converted to protein by multiplication by 6 25 after correction for non protein nitrogen (NPN)

The constituents of the serum proteins were measured by paper electrophoresis using a veronal buffer of pH 8 6 and ionic strength of 0 1

Theory

P | E

K_1

A_P C_P V_P | A_E C_E V_F | K_2 | C_L L

Fig 1

Fig 1 shows a two-compartment model with compartments P and E symbolizing plasma and extra vascular fluid compartments. According to the current concept of the transfer of protein across capillary membranes it is assumed that all protein filtered from P to E is carried away via the lymphatics

A_P = total amount of albumin in plasma compartment P
t = time
K_1 = coefficient of transfer from P to E (rate constant min)
C_P = concentration of albumin in compartment P
A_E = total amount of albumin in extra vascular compartment E,
C_F = concentration of albumin in extra vascular compartment E
K_2 = coefficient of transfer from E to outside
V_P = volume of P in heart capillaries corresponding to E,
V_F = volume of E drained by the lymphatic cannula
J_{PE} = flow of albumin from P to E
J_{FO} = flow of albumin from E
L = flow of lymph from E (g min)
C_L = concentration of albumin in lymph
A_P = amount of tracer I albumin in plasma compartment P
A_E = amount of tracer ¹³¹I albumin in extra vascular compartment E
C_P = concentration of tracer ¹³¹I albumin in P and
C_E = concentration of tracer ¹³¹I albumin in E

During steady state the flow of albumin from plasma to extra vascular fluid

$$J_{PE} = K_1 A_P \quad (1-1)$$

equals the flow of albumin from the extra vascular compartment drained by the lymphatic cannula

$$J_{FO} = K_2 A_E \quad (1-2)$$

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This amount of albumin has to be transported back to the plasma if a steady state is to be maintained In the lymph experiment however $J_{PE} = J_{EO}$ are small compared with the total amount of circulating albumin and a steady concentration of albumin is maintained for several hours

For a system of constant compartment size we can say

$$J_{IE} = J_{EO} = K \quad C_E \quad V_E \tag{1—3}$$

If the concentration of albumin in lymph adequately reflects that of the extra vascular fluid i e $C_E = C_L$ (steady state condition) we can say

$$J_{EO} = C_I \quad L = K_2 \quad C_E \quad V_E \tag{1—4}$$

Solving for V_E we get

$$V_E = \frac{L}{K} \tag{1—5}$$

Using the tracer I albumin we have

$$\frac{dA_E}{dt} = K \quad A_P^* - K_2 \quad A_E \tag{2—1}$$

If during the experiment A_P may be regarded as constant equation 2—1 becomes (cf Solomon 1953)

$$A_E = \frac{K}{K_2} \quad A_P^* \left(1 - e^{-K \quad t}\right) \tag{2—2}$$

and for $t = \infty$ we get

$$A_E - \frac{K_1}{K} \quad A_P \,(t = \infty) \tag{2—3}$$

$$\ln\left(1 - \frac{A_E}{A_{E\infty}^*}\right) = - K \quad t \tag{2—4}$$

$$K_2 = \frac{0\ 693}{T\ /} \tag{2—5}$$

In a system f con tant compartment si e $A_L = C_E \; V_E$ and

$$A_P = C_P \quad V_P \text{ and } \ln\left(1 - \frac{C_L}{C_{E\infty}}\right) = - K \, t \tag{2—6}$$

$$C_E = \frac{K}{V_E \quad K} \quad A_P \left(1 - e^{-K\,t}\right) \tag{2—7}$$

After a single inj ction of tracer I albumin into the plasma compartment the rise in the concentration of tracer in the lymph is determine l n serial samples (Fig 3) and $(1 - C_E \; C_{L\infty})$ is plotted against tim (cf Fig 4) g v ng the value of K (eq 2—6 2—5) V_E is no v obtain d from eq 1—5 and K from eq 2—3

The final value whi h C_L attains (cf Fig 3) d pends on the ratio K/K_2 (cf eq 2—3 and 2—7) but the *rate* at wh ch the system rea hes a teady state depends only on K e the rate of transport of albumin away from the extr vas ular space The values of K are alculated accord ng to eq 2 —3

In the present preparat on e are int rested in the plasm a volume of the capillaries corresponding to the extra vascular space drained by the lymphat c cannula (L) It s assumed that the total vascular volume of the heart mu cl rresp nds t of the heart weight (cf Pappenheimer 1953) The volume of the larg vessels has been estimated t be 0 9 of the heart we ght These estimates lead to a cap llary v lume f th heart equal to 1 6 of its weight Th s volume is then further reduced by th r ti f th drained extra vas ular albumin space to the total extra vascular album n space f the h rt Th total extra vascular album n space was assumed to be equal to the mannit l spa of th dog s heart i e 18 2 of the heart weight (Conn and Wood 1959)

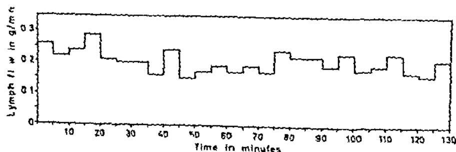


Fig 2 Lymph flow in g/min against time (min) in *HLP*

Results

After intravenous injection of [131]I albumin the test substance is *found* in the heart lymph within about 5 min *A dye* (Patent Blue or Evans Blue) *injected* into the central area of the left ventricle appears within approximately the *same* time Mixing seems *to* be quite rapid and there seem to be *only* insignificant differences here between appearance time and lag time *as reflected* in the rapid rise in the *concentration* of the test substance in heart lymph (cf Fig 3)

The results *of eight* successful experiments are shown in Table I and Fig 2 3 and 4 The cannulation of the heart lymphatics is somewhat difficult in dog heart lung preparations and could not be performed in two out of ten preparations

The flow of lymph from the heart varied between 0 018 and 0 041 g per *min* in the eight experiments (Table I) and could be kept quite *constant* for several hours in one and the same experiment (cf Fig 2) The lymph flow *could* be nicely controlled by changes of th hydrostatic pressure on he venous side *Increase* of this pressure was found to give up to sixfold *increase* of the lymph flow

The total protein *concentration* of the heart lymph was *found* to be quite high (2 1—? 7 g 100 ml) The concentration of albumin in *lymph* compared with that in plasma (C_L, C_P) varied between 0 66 and 0 83 (Table I The distribution of the various *proteins* in lymph and plasma was however *quite* similar i e all plasma proteins detectable by paper electrophoresis were also found in the heart lymph The albumin globulin *ratio in* lymph was about the same as that in plasma

Table I gives various parameters for the transport *of* [131]I albumin from the blood stream to the extra vascular space drained by the lymphatic cannula which have been calculated *according to* the two-compartment model described above

The total circulating *plasma* volume in the HLP varied between 410 and ? ml The volume of the extra vascular albumin space drained by th lymphatic cannula (V_L) showed values between 3 5 and 13 5 ml e 9—?? of the total extra vascular album n space of the heart tissue Any figure for th total extra vascular albumin space of the dog s heart has not been found in the literatur It as h r calculated equal to the mannitol space or 18 2 of the heart weight cf ? and Wood ? ?

The coefficient of transfer of albumin across the blood capillary walls K ari ? between 0 010 and 0 075 which means that about 1—8 of th albumin in V_P

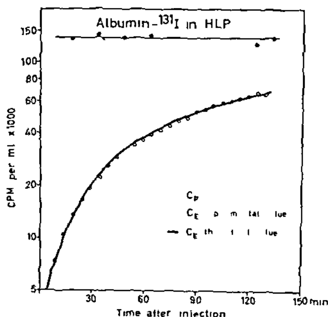


Fig 3 The concentration of I albumin in plasma (C_P) and heart lymph (C_L) in HLP plotted on a semilogarithmic scale against time The curved solid line has been drawn according to eq 2—7

TABLE I

Body weight (kg)	Heart weight (HW) (g)	Total extravasc alb space (18 2 % HW)	V_E (ml)	V_E in % of total extravasc alb space	k (min)	k_e (min)	C_L/C_P	Lymph flow (g/min)	1/k	Total plasma volume (ml)
12	78	14 2	4 7	33	0 048	0 0043	0 80	0 020	233	4 0
13	88	16 0	6 1	38	0 075	0 0067	0 83	0 041	149	4 0
14	95	17 3	9 0	52	0 072	0 0020	0 83	0 018	500	460
16	115	21 0	6 8	3	0 016	0 0050	0 82	0 034	200	410
17	105	19 1	9 2	48	0 034	0 0039	0 66	0 036	256	460
19	155	28 2	4 0	14	0 010	0 0075	0 81	0 030	133	572
25	139	25 3	13 5	53	0 010	0 0020	0 83	0 027	500	475
28	215	29 1	3 5	9	0 010	0 0116	0 78	0 010	86	715

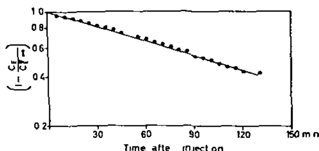


Fig 4 The removal of I albumin f om the extra vascular space via the lymphatic cannula The function $(1 - C_E/C_{E\infty})$ from eq 2—6 is plotted on a semilogarithmic scale against time The slope of the straight line drawn through the points is — K

crossed the capillary walls each minute The coefficient of transfer of albumin (K_2) away from the extra vascular space (V_E) via the lymphatics varied between 0 0020 and 0 0116 i e 0 2—1 2 of the albumin in V_E was carried away each minute via the lymphatics The turnover time or the average time the albumin molecule spent in the extra vascular space (1/K) was calculated as 257 minutes (mean value)

Fig 3 shows that the experimental values of the concentration of ^{131}I albumin in heart lymph are in good agreement with the theoretical values obtained from eq 2—7 If $(1 C_E/C_{E\infty})$ is plotted against time after injection a straight line is obtained (Fig 4) These results support the validity of the two-compartment model in the present experimental approach

Discussion

Some theoretical aspects and a model of lymph experiments in general have been discussed earlier by Grotte (1956) The main disadvantages in lymph experiments are

(1) The difficulty in appreciating the tissue area or volume that the lymph cannula is draining

(2) The variations of lymph flow

(3) The difficulty of an even approximate definition of lag time for comparison of concentrations of a test substance in plasma and lymph

(1) A review of the anatomy of the lymphatics of the dog s heart has been given by Drinker *et al* (1940) He concluded that this is probably the only organ in which all the lymph from one single organ could be collected when passing through one main lymphatic trunk at the base of the heart As shown by Allison and Sabiston (1957) however there are several lymphatics draining lymph from the dog s heart The larger trunk described by Drinker *et al* (1940) drains the main part of the l ft ventricle These anatomical observations by Allison and Sabiston have been confirmed by dye injection in our forty preparations (further experiments to be published)

The present results also confirm the view that functionally the total extra vascular space of the heart is not drained by a single lymphat c By using the tracer technique and applying the two-compartment model we obtained a quantitative estimate of the tissue volume drained by our lymphatic cannula We believe that in the present

investigation the difficulty mentioned above has been overcome and we may regard this lymph experiment as a quantitative study

(2) In the Starling preparations here reported it was possible to maintain quite steady pressures on both arterial and venous sides for several hours and accordingly the flow of lymph was remarkably steady as shown in Fig 2 In these experiments normal arterial and venous pressures were maintained and the results reported are only valid for the normal flow of lymph If the pressure on the venous side of the Starling heart lung preparation was increased the flow of lymph was also considerably increased The variations in lymph flow however depend upon a number of factors and for a recent review the reader is referred to Yoffey and Courtice (1956)

(3) The very rapid increase in the concentration of ^{131}I albumin in heart lymph after intravenous injection indicates that a small difference exists between appearance time and lag time In the calculations of Fig 3 and 4 lag time was assumed to be the same as appearance time i e instantaneous mixing in both compartments The application of the two-compartment model to the present experiments is on the other hand an over simplification where especially the assumption of instantaneous mixing in the two compartment model is not fulfilled in the strict sense The data however seem to show its usefulness as a working hypothesis and it gives a fair view of the kinetics of albumin within the dog s heart It gives an idea of the leakiness of the heart capillaries as regards protein molecules and of the normal transport of those molecules via the lymphatics

According to the model illustrated in Fig 1 it is assumed that no albumin passes directly from the extra vascular space back to the plasma i e all albumin is transported via the lymphatics It may very well be that some albumin also passes directly back to the plasma and that the C_l measured here only represents the net result of transport in both directions across the blood capillary membranes

The present results show that the blood capillary membranes of the dog s heart are quite leaky The steady state concentration of albumin in lymph was found to be as high as 66—83 of that of the plasma The similar distribution of proteins in lymph and plasma suggests that these proteins cross the capillary walls by a process of bulk flow rather than by diffusion which is in accordance with findings in other lymph experiments (cf Grotte 1956)

This study was supported by grants from the Swedish Medical Research Council the Swedish National Association against Heart and Chest Diseases Stockholm and Alice and Knut Wallenberg Foundation Stockholm Sweden The work first started at the Dept of Physiology of the University of Lund Sweden The stimulating help of Dr G Kahlson and associates and Baron T G Gyllenkrok is gratefully acknowledged

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Acta physiol scand 1964 62 218—223

From the Departments of Paediatric Surgery Plastic Surgery Physiology Clinical Physiology and Clinical Chemistry University of Uppsala Sweden

Studies on Heart Lymph

II Capillary Permeability of the Dog's Heart, using Dextran as a Test Substance

By

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Received 4 February 1964

Abstract

Areskog N H G Arturson G Grotte and G Wallenius *Studies on heart lymph II Capillary permeability of the dog's heart using Dextran as a test substance* Acta physiol scand 1964 *62* 218—223 — The capillary permeability of the normal heart has been studied in ten dogs using dextran as a test substance The capillary permeability has been determined as the ratio of the concentration of dextran of various molecular weights present in lymph and plasma under steady state conditions The results show a pattern similar to those of earlier investigations where lymph from the leg and the cervical region was collected i e capillary pores (pore radii 40—60 Å) and capillary leaks (120—300 Å) the only difference being an increased number of leaks in the heart capillaries compared with the other two regions

Only a few studies have been performed on the permeability of the blood lymph barrier of the heart In 1940 Drinker *et al* published experiments in which a lymphatic trunk from the heart of a dog was cannulated and various analyses of heart lymph were carried out They concluded that cardiac lymph is a filtrate from the blood capillaries it contains serum albumin and globulin and it clots Furthermore they showed that if horse serum was given intravenously it could be detected immunologically in the heart lymph and similarly gum acacia was also found in this lymph after intravenous injection

It appears reasonable to believe that the lymphatics of the heart play an important role in the normal and pathologic physiology of the heart This however is at present poorly understood and lacks confirmatory experimental evidence The aim of the present investigation was to measure the capillary permeability of the normal heart in dogs using dextran as a test substance

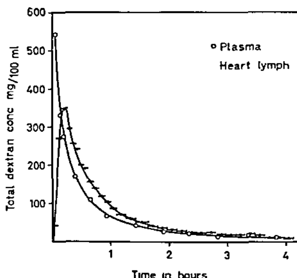


Fig 1 The total dextran concentration in plasma and heart lymph after i v injection of a sharp fraction of dextran ($\overline{M}_w$ = 5.200) 6 per cent in saline 10 ml/kg b w in a dog

Material and methods

Operative technique Ten young mongrel dogs weighing 15—25 kg and of both sexes were used Nembutal© anesthesia (veterinary Nembutal© Abbott 60 mg/ml) was used in all experiments For induction of the anesthesia 30 mg/kg body weight was given intravenously Small additional doses were given when required to maintain light anesthesia During the experiments mean arterial blood pressure pulse frequency and respiration were recorded The cannulation of the heart lymphatics has been described in detail elsewhere (Areskog Arturson and Grotte 1964)

In four of the experiments a modified dog lung preparation was used according to Areskog (196) and in three dogs both kidney pedicles were clamped to prevent kidney elimination of the test substance In the dogs given the sharp fraction ($\overline{M}_w$ 5 200) and the larger dextran molecules ($\overline{M}_w$ 180 000) the kidneys were left intact

The total concentration of dextran in serum and lymph was determined by the anthrone method (Wallenius 1954) The molecular weight distribution of dextran in each serum and lymph sample was estimated by turbidimetric titration (Wallenius 1954 Wallenius *et al* 1960)

Results

The dextran concentration curves for plasma and heart lymph obtained after a single intravenous injection of a fairly "monodisperse distribution (sharp fraction with a mean molecular weight of 5 200) are shown in Fig 1 The appearance time for dextran in heart lymph was found to be about 3—5 min The dextran concentration in the lymph then rose rapidly and reached ten minutes after the injection the same value as that in plasma Thereafter both curves showed a rapid fall with the heart lymph curve continuously lying above that of the plasma due to the rapid elimination from the plasma via the kidneys

After the injection of a dextran fraction of somewhat higher molecular weight distribution (mean molecular weight of 41 000 with the range m w 10 000—90 000) in a dog with occluded renal circulation the results obtained differ from those just described

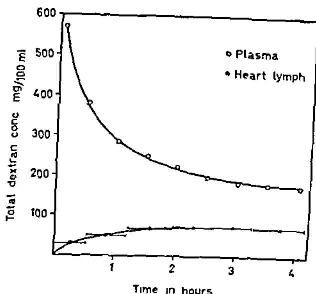


Fig 2 The total dextran concentration in plasma and heart lymph after i v injection of dextran with m w 10 000—90 000 6 per cent in saline 10 ml/kg b w in a dog with occlud d renal circula tion

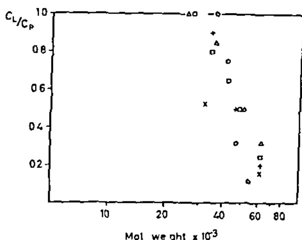


Fig 3 The lymph/pl a concentration ratio (C_L/C_P) plotted against mol cular weight after i v inj ction of dextran with m w 10 000—90 000 6 per cent in saline 10 ml/kg b w in four heart lung preparations (△ □) and in t c dogs w th occluded renal irculati (●)

g 2) As in previous studies of lymphatics draining other parts of the body (Grotte)6) the concentration of dextran in the lymph never reached that in the plasm but concentration ratio lymph/plasma (C_L/C_P) became constant with time The de ase of the dextran concentration in plasma and heart lymph is in this case quite slow
On the assumption that the system in Fig 2 is in a steady state the distributions of the various molecular sizes are compared in order to get an estimation of the mole cular restriction of the so-called blood lymph barrier in the heart The results of eight such experiments are shown in Fig 3 The ratio C_L/C_P rapidly fell with increasing sizes of the dextran molecules From the experiment shown in Fig 1 it was s en that

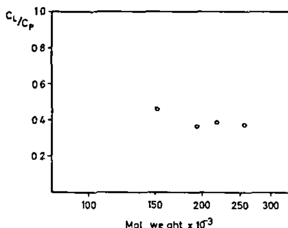


Fig 4 The lymph/plasma concentration ratio (C_L/C_P) plotted against molecular weight after i.v. injection of dextran with m w 100 000—350 000 6 per cent in saline 10 ml/kg b w in two dogs

C_L/C_P for dextran of molecular weight $\overline{M}_w = 5\,200$ reached unity or 1 0 during steady sieving across the membrane or membranes With increasing sizes of the molecules passing molecular weight from 20 000 to 60 000 an increasing restriction to their passage was found

In two further experiments still larger dextran molecules (mean molecular weight of 180 000 with the range 100 000—350 000) were injected Twenty four hours after the injection the concentrations of various molecular sizes in heart lymph and plasma were compared The results (Fig 4) showed that the concentration ratio (C_L/C_P) now became quite constant and independent of the molecular sizes of the test substance

Discussion

The heart preparation used in this investigation seems very suitable for studies of the blood lymph barrier The tissues drained are fairly homogeneous and the area fairly well known (Allison and Sabiston Jr 1957 Areskog *et al* 1964) The lymph flow is steady for several hours under standard conditions (Areskog *et al* 1964)

For the lymph sampling procedure a varying period of time will pass between the actual passage of a molecule through the capillary wall and the moment at which it can be collected at the end of the lymph cannula This sampling delay time or lag time can be evaluated approximately (Teorell 1937) Ideally the peak of the concentration curve in lymph should cross the curve of the plasma concentration This time difference illustrates the average lag time and can be estimated at about ten minutes in the present heart lymph experiments (Fig 1) This delay time will change with the rate of the lymph flow which however in the heart preparations used was constant during the experiment

The final outcome of the dextran concentration ratio (C_L/C_P) for the different molecular sizes used for determination of the sieving characteristics of the capillary membrane can be influenced not only by changes in the delay time but also by alterations in the plasma dextran concentration It will be seen from Fig 2 that the decrease of the plasma dextran concentration from about three hours after the injection of the dextran

preparation used and onwards is very slow and the system is in a steady state Determination of C_L/C_P under steady state conditions shows a pattern similar to those of earlier investigations where lymph from the cervical region and the leg of the dog was collected (cf Grotte 1956 Arturson 1961) Smaller molecules with a m w of about 5 000 quite rapidly equilibrate across the capillary walls The sieve coefficient i e the ratio C_L/C_P is unity or 1 0 For dextran molecules of somewhat larger sizes (m w 20 000—60 000) this ratio rapidly decreases (Fig 3) For the even larger molecules of m w 100 000—350 000 the ratio is constant at a fairly low value (Fig 4)

The restriction found for the passage of dextran molecules of the sizes of 20 000—60 000 may in accordance with the pore theory of capillary permeability be explained by the presence of capillary pores of at least the sizes of the passing molecules i e pore radii of 40—60 Å

Earlier investigations on the passage of dextran molecules across the capillary walls of the leg of the dog were interpreted according to the theory of restricted diffusions through capillary pores by Pappenheimer (1953) (cf also Grotte 1956 Arturson 1961) According to these investigations using widely different techniques capillary pores or water ways with approximate radii of 35—45 Å seem probable

The passage of molecules of higher m w (100 000—350 000) independent of molecular size may be explained by the presence of larger capillary leaks with radii of 120—300 Å (cf Grotte *et al* 1960) Investigations using electron microscopy also support this assumption (Bennet *et al* 1959) and the present investigation indicates the presence of these leaks in heart capillaries Similar leaks have been found in the leg and the cervical region but in the heart experiments higher C_L/C_P ratio for these larger molecules was found This may be explained either by an increase in the total number of the heart capillaries or of the number of leaks of them affecting the restriction curve for molecules of the sizes of 10 000—60 000 For both reasons this curve (cf Fig 3) will be shifted towards larger molecular sizes to the right

Thus it is possible that capillary pores are of the same sizes in the leg and cervical regions as in the heart the only difference being in the number of capillary leaks The C_L/C_P ratio for dextran molecules of the sizes of 100 000—350 000 as well as for the plasma proteins is quite constant for each region of the body (cf Yoffey and Courtice 1956 Grotte 1956)

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We wish to express our sincere gratitude to our laboratory engineer Mr Jo chim W [illegible] ky for excell nt technical aid especially concerning the molecular weight determin [illegible] of dextran

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The Metabolism of Fatty Acids in the Rat

I Palmitic Acid

By

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Abstract

Goransson G and T Olivecrona *The metabolism of fatty acids in the rat I Palmitic acid* Acta physiol scand 1964 62 224—239 — Data on the concentration and the fatty acid composition of neutral lipid fatty acids and of phospholipid fatty acids in blood liver heart kidney lung spleen and muscle from fasted and refed male rats are reported Data are also given on the radioactivity in these tissue lipid fractions at several time intervals after the intravenous injection of 9 10 H labeled palmitic acid in rat serum These data are intended as a reference for further studies on the metabolism of other long chain fatty acids The data are discussed with reference to a general picture of the metabolism of the plasma FFA after their removal from the plasma After entering the tissues the FFA are oxidized converted to other long-chain fatty acids esterified or transferred back to the plasma The fractions diverted into the different pathways depend upon the nutritional state The fraction oxidized is larger in the fasted than in the refed rats The diversion into one or the other of the possible pathways occurs almost immediately after the FFA have entered the tissue Subsequently the remaining labeled fatty acids are metabolized at considerably lower fractional rates since they have entered larger fatty acid pools than the plasma FFA pool

The plasma FFA represent the form in which fatty acids are mobilized from the fat depots and transported to other tissues (Fredrickson and Gordon jr 1958) The fate of the FFA after their exit from the plasma has been studied mainly by means of isotopically labeled fatty acids (Bragdon and Gordon jr 1958 Laurell 1959a Laurell 1959b Stein and Shapiro 1959 Borgstrom and Olivecrona 1961 Havel and Goldfien 1961 Olivecrona 1962a Havel Felts and van Duyne 1962) Most of these studies have concerned palmitic acid We intend to carry out a series of studies on the metabolism of the major long-chain fatty acids after the injection as albumin bound FFA into rats As a reference for these studies the present study of the metabolism of palmitic acid has been undertaken The aim is to follow in detail both the initial distribution and the subsequent metabolism of the labeled fatty acids Such studies have been carried out both in fasted and in refed rats to obtain a measure of the influence of the nutritional state on these

parameters It is apparent that any comparison of the metabolism of different fatty acids must involve a consideration of the fatty acid composition of the tissue lipids studied as well as the concentration of these lipids during the nutritional state studied We have therfore also included in the present paper data on these parameters

Methods

The experimental design was to give a group of rats an instantaneous tracer dose of H labeled palmitic acid Rats were then sacrificed at various time intervals and the radioactivity in their tissue lip ds stud ed. In other experiments the tissue lipid contents and the fatty acid compos t ons of these l p ds were stud ed

Preparat on of the injection solution 9 10-H palmitic ac d (The Radiochemical Centre Amersham England) was purified by reversed phase chromatography (Elovson 1964) twice and by subsequent TLC to assure that all radioactivity was in the form of unesterified palm tic acid. An aliquot of the labeled acid was taken to dryness redissolved in a minute volume of ethanol and an equivalent amount of KOH added to neutralize the fatty acid To this solution was added 50 volumes of fresh rat serum the mixture was allowed to stand at room temperature filtered and used immed ately On analys s more than 99 9 of the radioactivity was present in the FFA fract on.

Treatment of rats injection of the l beled serum and sampl The experimental animals were male Sprague-Dawley rats (del vered from AB Ant cimex, Stockholm) They were reared on a standard pellet diet and weghed 150—250 g at the time of the experiments The fasted rats received no food for 40 hrs prio to the experiment The refed rats were fasted for 24 hrs then given 20 glucose in 0 45 NaCl to drink *ad l b tum* for 14 hrs They were then g en 5 ml of the same solution by stomach tube and 1 hour later the labeled serum was injected

Under l ght ether anesthesia 0 5 ml of the serum contain ng the labeled palmitate was injected into the exposed right jugular vein The injection was ompleted in about 3 sec At appropriate t mes the rats were exsangu nated by withdrawing as much blood as poss ble f om the aort c b furcat on. The liver the heart the k dneys the spleen the lungs and in some experiments also samples from adipose t ssues and from muscles from d fferent anatomical locat ons were taken out All tissues were washed in wat r and mmediately transferred to chloroform methanol 2 1 The tissues were late homogen zed in the same sol ent the homogenate allowed to stand at room temperature som hours to ensure complete extrat on and then filtered into separat ry funnels conta n ng 0 4 volumes of KH PO and shaken After separation of the phases which usually equ ed 12 hrs the chloroform phase was dried over sodium sul f te and filtered into olumetric flasks This constituted the primary lipid extract from wh ch al quots were taken fo furth analys s

In some exper ments the blood corpuscular lip ds ere stud ed In these experiments clotting of the blood wa p e ented by the add tion of a small amount of sod um citrate and the blood wa centrifuged The plasma was withdrawn and the remaining blood corpuscles wa hed n 0 9 NaCl 5 times The blood corpuscles obta ned were then handled as described above for the tissues

In some experiment th arcass i e the rat m nus the organs taken out the skin or the whol body l p ds were studied The sample was then put in an E lenmever flask contain ng 100 g KOH 100 ml dist lled w ter and 300 ml eth nol The n ixture was then heated on a bo ling wate bath o rn ght The vol m was b ought to 1 000 ml w th ethanol and an al quot taken from wh ch th f tt cids were then xtra ted into petrc leum ether af er a d f cat on nd add tion of wat to th l quot Mod l experiment ha e hown that th eco ers f f tt ac ds using th pr cedur s bet er th n 95

A lyse perf rm d the mfl In most of the experiments the prim ry lip d e tra t w frac t onated on s l l lumns as pre ously described Ol ecrona 196 a I th e periments where the partial gl des n th l were tud ed the f llow ng p ecedure was used The p imary l p d extrac fr a whol l er was put on a 10 g sil c acid o umn n pet leu ther Hydroca bon ch les l esters and tr gl erides w re eluted w th 00 ml f 3 d h l ther n petroleum eth A f t n onta ing the d n l monoglycer des th free h lest ol nd the free fatty aci ls was th n l ted with chlorof rm F m this fra tion th f ee l les rol was then prec p tated th d t n de Cook 1958 Th remaining FFA d nd nonogl erid frac

TABLE I Tissue lipid concentrations and fatty acid compositions The lipid concentrations are acid compositions are expressed as percentage by area of the determined fatty acids

		Fasted rats							
		Lipid content	16 0	16 1	18 0	18 1	18 2	18 3	20 4
Liver	NF	60 ± 4 3	32 4 ± 1 7	2 8 ± 0 1	7 3 ± 0 8	27 3 ± 0 5	22 7 ± 1 7	1 8 ± 0 3	5 6 ± 0 4
	PL	490 ± 12	22 6 ± 0 6	—	30 7 ± 0 5	8 7 ± 0 5	15 5 ± 0 8	—	22 5 ± 0 4
Muscle	NF	2 300 ± 390	25 6 ± 1 8	5 4 ± 0 9	9 2 ± 0 6	38 7 ± 1 1	18 3 ± 1 5	2 8 ± 0 4	—
	PL	1 700 ± 180	29 6 ± 0 6	—	20 1 ± 2 1	11 3 ± 0 [illegible]	25 6 ± 1 3	0 2 ± 0	13 1 ± 0 5
Heart	NF	11 6 ± 1 1	27 5 ± 0 6	3 8 ± 0 3	12 6 ± 0 8	35 2 ± 1 0	17 1 ± 1 0	2 0 ± 0 1	1 8 ± 0 1
	PL	27 0 ± 2 8	14 5 ± 0 9	—	32 9 ± 0 6	10 3 ± 0 6	2[illegible] 1 ± 0 6	0 4 ± 0 1	19 7 ± 1 0
Kidneys	NF	21 7 ± 1 4	28 6 ± 0 6	2 8 ± 0 3	12 5 ± 0 2	29 6 ± 1 2	19 2 ± 0 5	2 5 ± 0 1	5 1 ± 0 5
	PL	66 4 ± 2 8	24 4 ± 0 2	—	24 1 ± 0 8	13 0 ± 0 3	12 6 ± 0 5	0 [illegible] ± 0	25 6 ± 0 1
Lungs	NF	10 9 ± 1 8	24 9 ± 0 5	3 4 ± 0 5	11 2 ± 0 8	36 4 ± 0 7	20 0 ± 0 8	3 1 ± 0 3	1 0 ± 0 2
	PL	29 5 ± 2 3	39 0 ± 1 8	—	19 0 ± 0 8	18 8 ± 0 6	8 4 ± 0 4	—	14 9 ± 0 6
Spleen	NF	3 1 ± 0 [illegible]	31 6 ± 1 6	4 1 ± 0 1	13 2 ± 1 1	32 1 ± 1 3	14 2 ± 1 8	1 9 ± 0 1	[illegible] 8 ± 0 8
	PL	7 1 ± 0 9	31 1 ± 0 7	—	20 4 ± 0 4	15 1 ± 0 7	1[illegible] 0 ± 0 [illegible]	1 3 ± 0 1	20 5 ± 0 6
Blood corpuscles	CE	—	41 [illegible] ± 1 1	5 9 ± 0 7	19 7 ± 0 2	17 4 ± 0 3	8 3 ± 0 8	—	7 6 ± 1 0
	FFA	—	41 1 ± 1 8	7 1 ± 0 9	22 1 ± 1 5	19 0 ± 1 2	5 3 ± 0 9	1 7 ± 0 3	3 7 ± 1 1
	GL	—	33 3 ± 1 2	11 6 ± 0 7	16 0 ± 1 3	23 5 ± 0 [illegible]	11 0 ± 1 2	4 4 ± 0 [illegible]	—
	PL	—	39 2 ± 2 1	—	22 6 ± 0 8	19 1 ± 0 8	8 5 ± 1 1	—	10 6 ± 0 4
Plasma	CE	—	13 1 ± 0 1	2 1 ± 0 3	2 4 ± 0 8	11 1 ± 0 9	17 0 ± 0 8	—	54 3 ± 0 5
	FFA	—	34 8 ± 2 3	7 5 ± 1 0	12 3 ± 1 0	25 2 ± 1 1	15 4 ± 1 [illegible]	2 3 ± 0 2	[illegible] 5 ± 0 4
	GL	—	2[illegible] 5 ± 1 [illegible]	2 9 ± 0 1	6 [illegible] ± 0 9	[illegible]4 9 ± 0 [illegible]	26 6 ± 1 1	2 5 ± 0 3	7 3 ± 0 3
	PL	—	36 1 ± 1 6	—	22 4 ± 1 6	11 ± 0 6	13 9 ± 2 9	0 8 ± 0 1	1[illegible] 0 ± 2 2

expressed as μEq per whole tissue and 200 g BW and are mean ± SEM of 6—9 rats The fatty and are mean ± SEM of 3 rats

Refed rats							
Lipid content	16 0	16 1	18 0	18 1	18 2	18 3	20 4
109	30 8	13 6	5 1	40 7	6 5	1 0	3 2
± 8 3	± 0 3	± 1 1	± 0 8	± 1.2	± 0 8	± 0 1	± 0 9
393	21 6	5 3	24 8	14 4	9 5	—	24 4
± 10 6	± 0 2	± 1 0	± 1 4	± 1 4	± 0 3		± 1 1
2 900	25 2	4 9	9 2	38 0	19 5	3 0	0 1
± 410	± 2 4	± 0 5	± 0 7	± 1 3	± 1 2	± 0 2	± 0
2 100	32 9	—	18 0	9 7	24 3	—	15 0
± 200	± 1 4		± 0 6	± 0 8	± 0 3		± 0 4
7 6	26 9	5 4	11 2	33 3	17 4	3 3	2 4
± 0 7	± 1 2	± 0 4	± 1 [illegible]	± 0 8	± 1 2	± 0 7	± 0 7
24 5	17 8	—	31 1	11 1	16 0	—	23 7
± 4 0	± 2 2		± 3 3	± 0 8	± 2 7		± 4 4
[illegible]5 0	30 9	3 9	14 2	29 8	14 0	2 3	4 8
± 4 3	± 0 5	± 0 4	± 1 0	± 1 3	± 0 7	± 0 1	± 0 7
76 9	24 [illegible]	—	24 5	1[illegible] 1	13 4	—	25 9
± 6 7	± 0 8		± 0 4	± 0 6	± 1 0		± 0 7
16 5	29 8	3 7	8 5	37 8	17 3	2 6	—
± 2 8	± 1 6	± 0 5	± 0	± 1 5	± 2 0	± 0 4	
23 7	41 0	4 4	15 8	16 7	6 8	1 0	14 2
± 1 1	± 1 8	± 1 0	± 0 6	± 0 2	± 0 5	± 0 1	± 1 6
4 [illegible]	35 3	5 6	19 5	24 [illegible]	8 6	2 1	4 2
± 0 6	± 1 0	± 0 3	± 1 7	± 0 4	± 0 7	± 0	± 0 5
12 3	3[illegible]	—	17 4	14 [illegible]	7 7	[illegible] 1	[illegible]3 1
± 1 7	± 1 7		± 1 6	± 0 6	± 0 7	± 0 4	± 1 2
—	30 5	12 1	19 1	21 8	12 1	—	4 3
	± 0 4	± 0 7	± 1 0	± 0 3	± 1 1		± 0 [illegible]
—	46 6	7 3	21 9	16 7	4 3	—	3 3
	± 0 9	± 0 6	± 0 [illegible]	± 0 6	± 1 0		± 0 4
—	33 8	10 9	15 4	26 7	11 [illegible]	—	2 0
	± [illegible]0	± 0 6	± 1 4	± 1 9	± 4 2		± 0 9
—	35 1	—	17 8	15 3	12 0	—	19 9
	± 2 4		± 1 2	± 0 9	± 1 [illegible]		± 0 1
—	13 5	7 0	4 2	16 1	9 4	—	49 7
	± 1 3	± 0 7	± 0 7	± 2 [illegible]	± 1 0	—	± 1 0
—	44 8	6 7	18 9	17 0	4 9	1 6	6 1
	± 2 1	± 0 8	± 1 4	± 1 5	± 0 8	± 0 3	± 0 3
—	[illegible]1	11 6	4 7	35 4	[illegible]	3 8	5 5
	± 0 8	± 1 5	± 0 4	± 0 [illegible]	± 1 8	± 0	± 0 [illegible]
—	33 [illegible]	6 9	16 9	15 3	8	5	16 6
	± 0 7	± 0 6	± 0 8	± 1 1	± 1 1	± 0 6	± [illegible]3

tions were separated on TLC using Silica gel G (Merck AG Germany) and a solvent system of acetic acid methanol diethyl ether heptane 1 1.5 10 40. The plates were sprayed with iodine in methanol, and the respective spots scraped off and eluted with chloroform into 25 ml volumetric flasks.

In some preliminary experiments the phospholipid fractions from the tissue lipids were further fractionated on TLC (Skidmore and Entenman 1962a, Skipski Peterson and Barclay 1962). The spots were identified as described by Skidmore and Entenman (1962a). To determine the radioactivity in the phospholipid fractions unstained spots were scraped off and suspended in a *thixotropic gel prepared from toluene scintillating substances* and Cab-O Sil (Packard Instrument Company Inc., La Grange, Ill., U.S.A.) as described by Snyder and Stephens (1962).

Esterified fatty acid content in the lipid fractions was determined using the method of Skidmore and Entenman (1962b). *Cholesterol esters were determined by the method of Webster* (1962). FFA were dissolved in ethanol and titrated with 0.02 N NaOH in methanol using nile blue as indicator. Radioactivity was determined by liquid scintillation counting as previously described (Olivecrona 1962b).

The fatty acid composition of the tissue lipid fractions was determined by GLC. The primary lipid extracts were separated by silicic acid chromatography into a cholesterol ester fraction which was discarded, a glyceride plus FFA fraction, and a phospholipid fraction. The two latter fractions were transmethylated by refluxing in 2% H_2SO_4 in methanol overnight at 60 C. The methyl esters and the free cholesterol *were extracted into petroleum ether and put on to a* silicic acid column, from which the methyl esters were subsequently eluted with 2% ether in petroleum ether while the cholesterol was retained on the column. In preparing and storing the methyl esters special care was taken to avoid auto-oxidation of the unsaturated fatty acids. The methyl esters were analyzed with a Pye Argon Chromatograph which was equipped with a β-ray ionization detector. Column packing consisted of 20% ethylene-glycol succinate on acid washed Celite (100—120 mesh). The acids were identified by comparing their retention times with those of known standards. The amounts of the respective fatty acids were determined with a Pye integration amplifier. The determined amounts of 16 0 16 1 18 0 18 1 18 2 18 3 and 20 4 were taken as 100% and the amounts of the individual fatty acids reported as fractions of this. The designation of the fatty acids gives number of carbon atoms and double bounds (Farquhar *et al.* 1959).

Results

Tissue lipid concentrations and fatty acid compositions. Table I. The neutral lipid fraction of the tissue lipids contained mainly glyceride fatty acids. In addition cholesterol ester fatty acids and FFA were included in this fraction. A separate experiment showed that in all tissues but the blood, cholesterol ester fatty acids represent less than 2% of the total esterified fatty acids in the "neutral lipid" fraction. Therefore the cholesterol esters were not separated from the neutral lipid fraction when determination of the concentration of esterified fatty acids in this fraction was performed. In the analysis of the fatty acid composition of the neutral lipid fraction, *however, the cholesterol esters* were separated and discarded, so that the fatty acid composition reported is that of the glyceride plus free fatty acids.

All tissues but the adipose tissue and the muscles contained more phospholipid fatty acids than neutral lipid fatty acids. It is conceivable that the relatively high content of neutral lipid fatty acids in the muscles was due to the presence of intermuscular adipose tissue. The content of neutral lipid fatty acids tended to rise in most tissues with refeeding, this tendency being most marked in the liver. The phospholipid content of the tissues did not change with the nutritional state. The total body fatty acid content was 31 ± 5 and 28 ± 2 meq per 100 g b. w. in the refed and fasted rats, respectively. It was observed that starting with the same body weight refed rats tended to have a slightly higher weight than fasted ones at the time of sacrifice. The differences was of

TABLE II Blood lipid concentrations and radioactivity in whole blood and blood corpuscular lipids 20 min after the intravenous injection of H palmitic acid in rat serum The lipid concentrations are expressed as μEq of fatty acid per ml of blood and are mean ± SEM of 6 rats The radioactivity is expressed as percent of the injected radioactivity present in the whole blood volume which was taken as 8 g per 100 g BW (Mean of 2 rats)

	Fasted rats			Refed rats		
	Whole blood		Blood corpuscles	Whole blood		Blood corpuscles
Lipid fraction	Lipid conc.	Radioactivity	Radioactivity	Lipid conc	Radioactivity	Radioactivity
Cholesterol esters	0 54 ± 0 03	0 011	0 0007	0 32 ± 0 03	0 53	0 002
Free fatty acids	*0 54 ± 0 02*	*0 17*	*0 008*	*0 19 ± 0 05*	*0 13*	*0 007*
Glycerides	0 39 ± 0 03	0 58	0 02	0 61 ± 0 07	0 15	0 05
Phospholipids	2 60 ± 0 06	0 08	0 06	2 50 ± 0 16	0 23	0 12

the order of 10—15 g in 200 g rats The tissue lipid contents when referred to the body weight were therefore divided by a somewhat higher factor in the refed than the fasted rats

In a separate experiment the partial glycerides and the FFA in the liver were separated on TLC and their concentrations determined In the refed state these concentrations were diglyceride fatty acids 3 2 ± 0 3 monoglyceride fatty acids 1 2 ± 0 2 and FFA 4 8 ± 1 1 μeq per whole liver and 200 g b w (mean of 6 rats)

Some general tendencies with regard to the distribution of the fatty acids were found 16 0 was a major constituent in all the lipid fractions making up 12—49 % of the fatty acids in the fractions studied 16 1 was present mainly in the glycerides usually contributing less than 10 % of the fatty acids in this fraction It showed a tendency to rise in the refed state the tendency being most marked in the liver glycerides 18 0 was present mainly in the phospholipids but also in lesser proportions in the glycerides 18 1 was present both in the glycerides and in the phospholipids but always in a higher proportion in the glycerides 18 2 was present in considerable amounts in both the glycerides and in the phospholipids 18 3 was found in small amounts in the glycerides in most tissues 20 4 was present in high proportions in the phospholipids in most tissues but was a very minor constituent in the glycerides In general the phospholipids contained a higher proportion of unsaturated fatty acids than the glycerides and in both fractions there was a change towards a higher proportion of satured fatty acids in the refed state

The glyceride fatty acids showed a relatively similar composition in all tissues but the phospholipid fatty acid composition was more variable between the tissues On the other hand there were more marked changes in the fatty acid composition of the glycerides than of the phospholipids with the nutritional state

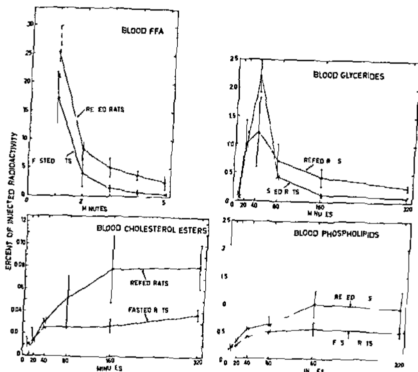


Fig 1 Radioactivity in blood lipid fractions in rats after the intravenous injection of H palmitic acid in rat serum *The values are given as percent of the injected radioactivity present in the* whole blood volume which was taken as 8 g per 100 g B W The FFA radioactivity values are mean ± 3 SEM of 9 rats the other values are mean ± 3 SEM of 6 rats at each time

The plasma glycerides had a fatty acid composition similar to that of the liver glycerides and the change with the nutritional state was also the same in both fractions Thus in the refed rats there was a much higher content of 16 1 in both the liver and the plasma glycerides than there was in the fasted rats The plasma phospholipids on the other hand were different in their fatty acid composition from the liver phospholipids The change in fatty acid composition with the nutritional state however showed some similarities between the liver and the plasma phospholipids Thus in both fractions there was a characteristic rise of 16 1 and a decrease of 18 2 in the refed rats The plasma cholesterol esters were characterized by a very high content of 20 4 whereas the blood corpuscle cholesterol esters contained only minor amounts of 20 4 The plasma FFA in the fasted rats showed a fatty acid composition similar to that of many of the tissue glyceride fractions in this state In the refed rats the plasma FFA had changed their fatty acid composition markedly towards a higher degree of saturation and contained high proportions of palmitic and stearic acid The FFA of the blood corpuscles had the same fatty acid composition in both nutritional states This was similar to the fatty acid composition of the plasma FFA in the fasted rats

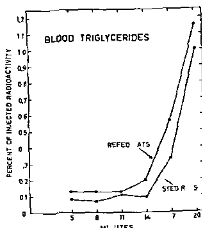


Fig 2 Rad oactivity in the bl od trigl ceride frac tion in rats after the intravenous injection of H palm tic acid in rat se um The values are expressed as percent of the injected rad oactivity and are mean of 3 rats

Blood lipid concentration and radioacti ity

The lipid content of whole blood is shown in Table II The concentrations of cholesterol ester fatty acids and of FFA were higher in the fasted than in the refed rats The con centration of glyceride fatty acids on the other hand was higher in the refed rats The phospholipid fatty acid concentration did not change much with the nutritional state The high phospholipid fatty acid concentrations were probably due to a high concen tration of this lipid fraction in the blood corpuscles

The distribution of radioactivity between the blood plasma and the blood corpuscles 20 min after the injection of the labeled palmitic acid is also shown in Table II There was very little radioactivity in the blood corpuscle cholesterol esters glycerides or FFA indicating that radioactivity in these fractions was present almost exclusively in the blood plasma The blood corpuscle phospholipids on the other hand contained most of the phospholipid radioactivity of whole blood in the fasted rats and almost half of the whole blood phospholipid radioactivity in the refed rats

The radioactivity in the blood lipid fractions is show n in Fig 1 and 2 At 2 min there was only 8 % and 3 of the injected radioactivity left in the blood FFA in the fasted and refed rats respectively If it is assumed that the di appearance of the radioactivity during the first 2 min followed a first order exponential then the fractional turnover rates (Zilversmit 1960) for this disappearance can be calculated to be 1 2 and 1 5 per min in the fasted and the refed rats respectively The radioactivity in the blood glyc eride fraction was low at 5 and 10 min then rose to a maximum at 40 min and de clined again. The maximum was higher in the fasted than in the refed rats but the general course of the curve was similar in the tw o nutritional states To study in more detail the appearance of radioactivity in the blood tr glycerides a separate expe iment was carried out in which frequent blood samples were drawn from rats 5—20 min after injection of the labeled palmitic acid The results ar sho n in Fig 2 which shows mean values from 3 refed and 3 fasted rats The rad oactivity was low up to 14 min and then started to rise steeply between 14 and 17 min both in the fasted and in the refed rats The radioactivity in the blood phospholipid fraction showed a slow gradual rise during the first 160 min and essentially similar values at 320 as at 160

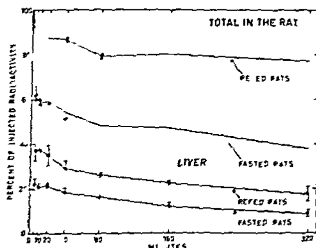


Fig 3 Radioactivity in the liver and in the whole body in rats after the intravenous injection of H-palmitic acid in rat serum. The values are expressed as percent of the injected radioactivity. The data on total represent the sum of the radioactivity in the tissues removed and in the carcass or the radioactivity found in extracts from digested whole rats. They represent data from single rats. The liver data are mean ± 3 SEM of 6 rats at each time.

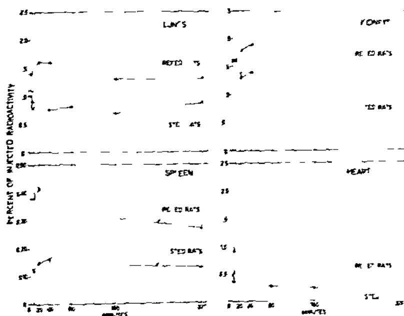


Fig 4 Radioactivity in the lungs, the kidneys, the spleen, and the heart in rats after the intravenous injection of H-palmitic acid in rat serum. The values are expressed as percent of the injected radioactivity and are mean ± 3 SEM of 6 rats at each time.

min. The values were lower in the fasted rats but the course of the curve was similar as in the refed rats. The cholesterol ester radioactivity was much less than the glyceride or phospholipid radioactivity and the curve has therefore been drawn in a different scale. The radioactivity in this fraction rose gradually during the entire time period studied and, like the phospholipid radioactivity, the values were lower for the fasted rats.

TABLE III Radioactivity in liver lip d fractions and in carcass lipids in rats after the intra venous injection of H palmitic acid in rat serum The values are expressed as percent of the injected radioactivity The upper 10 values represent data from single rat the lower 7 values are mean ± SEM of 6 rats

Min after injec tion	Liver		Carcass	Liver		Carcass
	Neutral lipids	Phospho- lip ds	Total lipids	Neutral lipids	Phospho- lipids	Total lipids
1	8 6	5 6	63	13 4	10 2	—
	8 6	6 5	64	15 5	9 4	—
2	*8 3*	*10 4*	*52*	*15 8*	*14 0*	—
	9 0	7 3	50	16 9	14 8	—
3	14 2	11 6	48	15 4	13 7	—
	—	10 2	48	17 1	—	—
4	9 8	7 2	48	21 5	15 3	—
	10 6	7 4	48	11 9	17 3	—
5	11 3	8 4	47	11 3	19 0	63
	10 7	6 9	46	12 2	16 6	63
5	9 3 ± 0 2	10 1 ± 0 3		15 6 ± 0 4	17 9 ± 0 3	
10	8 9 ± 0 1	9 3 ± 0 3		15 5 ± 0 5	17 8 ± 0 4	
20	8 9 ± 0 2	13 4 ± 0 7		10 8 ± 0 4	19 4 ± 0 7	
40	5 0 ± 0 1	12 3 ± 0 3		9 2 ± 0 4	17 4 ± 0 4	
80	3 2 ± 0 1	12 3 ± 0 3		6 5 ± 0 2	17 6 ± 0 2	
160	1 5 ± 0 04	11 5 ± 0 4		6 2 ± 0 4	15 3 ± 0 7	
320	0 9 ± 0 04	7 0 ± 0 2		5 0 ± 0 4	11 4 ± 0 5	

Tissue radioactivity (Fig 3 and 4) *The total lipid soluble radioactivity decreased* rapidly in the fasted rats and was only 63 at 5 min In the refed rats on the other hand more than 90 % could be recovered at 5 min From 5 min on the decline of the total lipid soluble radioactivity occurred slower and the mean values at 320 min were 74 % and 35 % in the refed and fasted rats respectively

There was initially more label in the livers of the refed rats than in the livers of the fasted ones and in both groups the radioactivity then declined to about half its value at 320 min (Fig 3) The radioactivity in the liver neutral lipid and phospholipid fractions is shown in Table III Initially the neutral lipid fraction contained more radioactivity than the phospholipid fraction From 2 min on the phospholipid radioactivity continued to rise whereas that in the neutral lipid fraction remained unchanged so that the phospholip d radioactivity soon became higher than the neutral lipid radioactivity From 10 or 20 min on the radioactivity in the liver neutral lipid fraction decreased and later the radioactivity *in the phospholipid fraction also declined However* the livers contained more phospholipid than neutral lipid so that the specific radioactivity in the two fractions were similar at the end of the experiment (Table VI) Radio-

TABLE IV Radioactivity in liver tri di and monoglycerides FFA and phospholipids in refed rats after the intravenous injection of H³ palmitic acid in rat serum The values are expressed as percent of the injected radioactivity

Min after injection	Triglycerides	Free fatty acids	Diglycerides	Mono glycerides	Phos pho lipids
1	11 8	0 32	6 5	0 21	10 3
3	16 7	0 10	4 9	0 35	14 7
3	16 1	0 15	4 1	0 32	15 4
8	15 2	—	2 9	—	17 5
20	16 3	0 11	0 7	0 17	17 7
20	13 8	0 11	0 5	0 17	17 ?

TABLE V Radioactivity in adipose tissue and *muscle fatty acids in rats after the intravenous* injection of H palmitic acid in rat serum The weights are expressed in gram and the radioactivity as percent o the injected radioactivity Mean ± SEM of 3 rats The adipose tissue radioactivity is that of the skin lipids and the easily d sected abdominal cavity adipose tissue The muscle radioactivity was calculated using th specific radioactivity of abdominal wall or hind limb muscle and the assu ption that 45 of the body weight was muscle tissue

	Fasted rats		Refed rats	
	5 min	320 min	5 min	320 min
Body weight	195 ± 2	193 ± 1	228 ± 3	273 + 8
Adipose tissue fatty acids	3 5 ± 0 1	? 3 ± 0 1	4 ? ± 0 5	4 4 ± 0 3
Adipose tissue radioactivity	2 ± 0 3	2 8 ± 0	6 7 ± 0 4	6 9 0 7
Muscle abdominal wall radioactivity	6 ? ± 1 0	6 7 ± 1 0	14 ? ± 2 0	9 6 ± 0 6
Muscl hind limb radioactivity	12 8 ± 0 8	8 0 ± 1 0	17 5 ± 2 5	15 3 ? 4

activity in the liver triglyceride diglyceride monoglyceride FFA and phospholipid fractions at 1 3 8 and 20 min after the injection of labeled palmitic acid is shown in Table IV Most of the radioactivity in the n utral lipid fraction was found in the tri glyceride fraction whereas the monoglyceride and the FFA fractions showed low levels of radioactivity at all times studied *The diglyceride radioactivity was highest* at 1 min when it was about half of that in the triglyceride fraction The diglyceride radioactivity *then decreased whereas the triglyceride radioactivity rose from 1 to 3 min* and then remained essentially unchanged up to 20 min

The radioactivity in the heart the spleen the kidneys and the lungs is shown in Fig 4 In the heart the lungs and the spleen the radioactivity at 5 min *was less in the fasted rats* than in the refed rats In the kidneys these levels of radioactivity were similar In the refed rats the radioactivity continued to rise from 5 to 20 or 40 min *in both the*

TABLE VI Specific radioactivity of tissue lipids in rats after the intravenous injection of H palmitic acid in rat serum The values are expressed as percent of the injected radioactivity per mEq of fatty acid and are mean of 3 rats

		Fasted rats		Refed rats	
Organ	Min after injection	Neutral lipids	Phospholipids	Neutral lipids	Phospholipids
Liver	2	142	19	146	35
	320	15	15	45	29
Muscle	2	6	2	7	3
	320	2	0 5	3	0 5
Heart	2	23	47	197	81
	320	7	7	47	5
Kidneys	2	61	12	36	18
	320	9	7	5	10
Lungs	2	30	15	31	41
	320	24	7	14	2
Spleen	2	90	5	31	4
	320	5	6	11	7

kidneys and in the lungs In the heart the radioactivity remained essentially constant from 5 to 20 min and then declined In the spleen there was a rise from 5 to 20 min then a decline followed by a second rise to a maximum at 80 min from which the radioactivity then declined In the fasted rats the radioactivity declined after 5 min in the heart the kidneys and the lungs but in the spleen it rose to a second maximum at 80 min i e the same time as the maximum in the spleens of the refed rats

Adipose tissue and muscle radioactivity is shown in Table V These tissues can not be sampled *in toto* and any particular sample studied is not necessarily representative for the entire organ There was considerable variation in the values for muscle radioactivity obtained from different samples The values indicates that the muscle radioactivity was higher in the refed than in the fasted rats both at 5 and at 320 min The fatty acids in the adipose tissue sample studied represented 1/3 to 1 2 of the total fatty acids in the rats Thus the total adipose tissue radioactivity was certainly higher than that shown in table 5 by a factor of 1 5 or 2 In separate experiments we have studied the specific radioactivity in adipose tissue from different locations Consistently the retrobulbar fat tissue had the lowest specific radioactivity the epididymal fat pad was usually low whereas the subcutaneous and the mesenteric adipose tissue often showed considerably higher specific radioactivity

In most tissues the neutral lipids had a higher specific radioactivity than the phospholipids at 2 min (Table VI) In all fractions but the spleen phospholipids the specific radioactivity decreased from 2 to 320 min In some but not all tissues the specific radioactivity in the neutral lipids and in the phospholipids were more similar at 320 than at 2 min

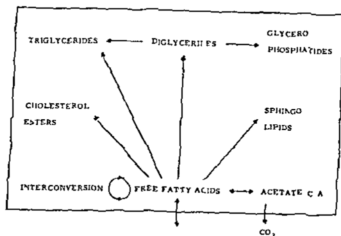


Fig 5 A hypothetical scheme for the metabolism of plasma FFA after their entry into a cell

Discussion

The present study has been undertaken to establish a reference for projected studies of other long chain fatty acids after injection as albuminbound FFA To obtain such a reference it has been necessary to repeat some older work on the metabolism of injected palmitic acid under our conditions Thus much of the present data serve to reinforce and extend the findings of previous studies Since the matabolism of fatty acids is highly dependent upon the nutritional state we have carried out our experiments using rats in two quite different nutritional states In the fasted state the fatty acid metabolism is geared to provide substrate for oxidation by the tissues and there is a large net flux of FFA from the adipose tissue to other tissues In the carbohydrate fed state in this work the refed rats there is much less net flux of FFA from the adipose tissue to other tissues only a small fraction of the FFA is oxidized and fatty acids are synthesized from nonlipid sources to replenish the adipose tissue stores

It is well known that the plasma FFA fraction has a high fractional turnover rate and that injected labeled FFA rapidly disappear from the circulating blood (Fredrickson and Gordon Jr 1958) Their fate after entry into the cells is less well known Fig 5 is a hypothetical scheme of the pathways open to the FFA upon entry into a cell The present data show that the labeled fatty acids were shunted into one or the other pathway almost immediately after their entry into the cell and it is apparent that it is during this phase that we would expect to find the most marked differences between different fatty acids

Oxidation In the present experiment the total radioactivity recovered in lipid form was studied at a series of time intervals Both in the refed and in the fasted rats this fraction decreased with time indicating oxidation of the labeled fatty acid The decrease occurred more rapidly in the fasted than in the refed rats which is in agreement with many previous studies that show that carbohydrate spares the oxidation of fatty acids (McCalla Gates and Gordon Jr 1957 Fritz 1961) In the fasted rats the decrease occurred very rapidly during the first 5 min particularly during the first 2 min This is in good agreement with the view that plasma FFA are directly available for oxidation In the refed rats on the other hand more than 90 % of the radioactivity could be

recovered in lipid form at 5 min Thus only a small fraction of the FFA entering the tissues in this nutritional state is oxidized immediately Subsequently there was a continued decrease in the lipid soluble radioactivity indicating continued oxidation The rate of this continued oxidation was more rapid in the fasted than in the refed rats but the difference in rate between the two groups was much less marked than during the first 5 min. The possibility exists that the major difference in fatty acid oxidation between fasted and refed rats is that a larger fraction of the FFA coming into the tissues is oxidized in the fasted rats The tissue lipids may be drawn upon for oxidation within the tissues at more similar rates in the two nutritional states

In all tissues but the kidney there was less radioactivity 5 min after the injection in the fasted rats than in the refed rats It is possible that this was due to the more rapid oxidation of the FFA in the fasted rats If in addition differences exist in the fractions of the plasma FFA taken up by the individual tissues in the two nutritional states can not be decided from the present data

Formation of other long-chain fatty acids Palmitic acid is not converted to other long chain fatty acids to any large extent in fasted rats, but in refed rats such conversion is extensive (Elovson paper in preparation) Thus in the refed rats considerable fractions of the label in the tissue lipids will be in C_{16} monounsaturated and C_{18} saturated and monosaturated acids and not only in palmitic acid so that the data obtained in the refed rats can not be considered strictly representative for palmitic acid metabolism

Esterfication of the FFA In all tissues studied the label was rapidly incorporated into both the neutral lipid and the phospholipid fraction In most tissues the specific radioactivity of the neutral lipid fraction was considerably higher than that of the phospholipid fraction at 2 min, indicating a more rapid fractional turnover rate for the neutral lipids According to Kennedy (1961) both the triglycerides and the glycerophosphatides are formed from a common intermediary the diglycerides The finding that the liver diglyceride radioactivity rose rapidly and then decreased rapidly again whereas both the triglyceride and the phospholipid radioactivity continued to rise after the diglyceride radioactivity had started to decrease is consistent with the view that the diglycerides are intermediates in the formation of the triglycerides and of the glycerophosphatides

In addition to the glycerides and the glycerophosphatides discussed above labeled fatty acids can also be incorporated into cholesterol esters sphingolipides and other not glycerol-containing lipids Chemical studies of the tissue content of cholesterol esters showed that they represent less than 2 % of the total neutral lipid fatty acids In some experiments the radioactivity of the liver cholesterol esters was studied and it was found that less than 0 1 % of the injected radioactivity was located in this fraction 20 min after the injection of the labeled palmitic acid In all tissues the cholesterol esters have been included in the neutral lipid fraction The phospholipid fraction was further fractionated on TLC in some preliminary experiments and more than 80 % usually more than 90 of the radioactivity in this fraction was found in glycerophosphatide fractions 2 min after the injection of the labeled palmitic acid Thus most of the label in all tissues was associated with either glyceride or glycerophosphatide fractions

The fatty acid composition of both glycerides and phospholipids in all tissues changes toward a higher content of saturated fatty acids in the refed state This was true also for the plasma FFA fraction It is conceivable that the change in the composition of the plasma FFA may have been a major cause of the change in the tissue lipids since the plasma FFA are a major precursor of these lipids On the other hand it is also possible that the changes in plasma FFA and tissue lipid fatty acid composition are a reflection

of the same basic change which could be that the major source of fatty acids in the refed state is synthesis of fatty acids from nonlipid sources and that the fatty acids synthetized are predominantly saturated fatty acids

Transfer of fatty acid esters to the plasma Label rapidly appeared in the plasma glycerides and to lesser extent in the plasma phospholipids and cholesterol esters Previous studies indicate that the fractional turnover rates of plasma triglycerides are higher than for plasma phospholipids and cholesterol esters (Bates 1958) This is in agreement with the present results which show a more rapid and extensive labelling of the plasma triglycerides than of the plasma phospholipids or cholesters esters and also a more rapid decrease of the plasma triglyceride radioactivity An interesting finding was the time lag before radioactivity appeared in the plasma triglycerides Such a time lag has been reported by several authors previously to be present in several animal species (Laurell 1959b Stein and Shapiro 1959 Borgstrom and Olivecrona 1961 Havel *et al* 1962) In these experiments we found that the radioactivity started to rise steeply between 14 and 17 min The time lag was the same in the fasted and the refed rats Since the liver triglyceride reached their maximal radioactivity rapidly the time lag must occur in the transfer of the triglyceride from the liver to the plasma Possibly it reflects the time necessary for the assembly of the lipoproteins from their individual parts

It seems well established that in the fasted state plasma triglyceride and phospholipid originates in the liver (Frederickson and Gordon Jr 1958 Borgstrom and Olivecrona 1961 Havel and Goldfien 1961 Fisher *et al* 1943 Goldman *et al* 1950 Harper Jr Neal Jr and Hlavacek 1953 Olson 1958) The data on the fatty acid composition of the liver and the plasma triglyceride show that these were quite similar Furthermore in both fractions there occurred the same characteristic changes with the nutritional state These results lend additional evidence to the view that the liver is the major source of endogenous plasma triglyceride The liver and the plasma phospholipids on the other hand showed considerable differences in their fatty acid composition in the two nutritional states However the changes with the nutritional state occurred in the same directions in both the liver and the plasma phospholipids with a characteristic increase in the proportions of palmitoleic and oleic acid and a decrease in the proportions of stearic and linoleic acid

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Miss Irene Kalebsson gave skilful techn cal as istance

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longer period of time have been reported (Asmussen 1940 Cobb and Johnson 1963) and there have been none dealing with the circulatory and respiratory changes during non steady state exercise

A steady state is generally considered to be present when there are no mea ureable changes in the circulation respiration and metabolism within a defined period of time The parameters usually used to demonstrate the existence of steady state are oxygen uptake RQ ventilation respiratory rate heart rate and lactic acid concentration in arterial blood It is obvious that the steady state of these parameters can only be maintained for a certain period of time whose duration will depend on such factors as work load metabolic state and the degree of fitness of the subject It is therefore probably better to discuss a relatively steady state and to define the period of time covered and the variables included

During non steady state work we should thus expect the abovementioned parameters to change with the time factor and at a rate depending on the same factors as those determining the individual s ability to maintain a steady state

The present investigation was planned to study the cardiorespiratory changes during exercise at a work load selected individually to produce a situation where the changes in circulation, as judged by the change in heart rate occurred slowly

The circulatory response was studied with heart catheterization technique and the respiratory changes were studied with the aid of arterial blood and expired air samples

Material

Six healthy male sub ects volonteered for the study They were all phys cally active but none regarded himself as being in good trim All were blood donors registered at and controlled by the hospital blood bank Anthropometric data and some data concerning the dimensions of the vascular system are g ven n Table I Prior to the investigation all the subjects underwent a clin cal examination including chest X ray ECG at rest and during work They sho ed no signs of disease

Methods

The *elect oc d ram* at rest and during work has been described n deta l earlier (Holmgren and St andell 1961)

O tho tatic test Heart rate and ECG were recorded after eight minutes standing in th erect position

The *wo k capac ty* was determined as th amount of ork the subject could perform on a b cycle ergometer (Hol ngr n and Mattson 1954) at a heart rate of 170 beats p r m n (W) (Sjostrand 1947) The procedure was the sa ne as has been described ea l er (Holmgren Jo sson and Sjostrand 1960)

The *t tal amount of hemo l bin* (THb) was determin d with the al eolar CO m thod (Sj strand 1948) with the mod fication int oduced by W klunde (1956) and with a reproducib lity of four per cent

The *blood vol me* was calculated f om THb nd the haemogl bin con entration in arterial blood in the sup ne po tion thus disregard ng the rror nt oduced th ough the assumpt on of a const nt hemoglobin con entrat on in the whole blood volume During long t rm ex ise the blood volume was det rm ned with I labell d lbumin as the 10-min I sp ce (co ected f the d ff rence between body ha natocrit and larg ess l HCT f t r 0 91) w th the aid of th sem autom tic dev ce called Volumetron (Atomium Corp Waltham Mass U S A) and w th a reproduc b l ty of 2 9 per cent

The *he t ol m* was d term ned n the pron pos t on cording to the method descr bed by Larsson and Kj llberg (1948 with a repr duc bil ty of four per cent

R ght hea t cath ter tion was perf rmed with con ent onal techn que from the l ft arm with a number 9 doubl l m en catheter tip The ight brach al artery was cath terized percutaneously (Bernéus et al 1954) w th a nylon fish ng line leader and teflon catheter

TABLE I. Anthropometric data in six healthy young men

Case no	Age years	Height cm	Weight, kg	Heart vol. ml	THb g	Hb con. g/100 ml	Blood vol. l	W_{170} kpm/min supine	W kpm/min sitting
2	25	184	83	[illegible]0	990	13.3	6.8	1,400	1,[illegible]00
3	20	190	[illegible]0	745	890	14.7	6.1	1,200	1,350[1]
4	20	184	69	[illegible]0	800	13.5	5.9	1,100	1,0[illegible]0
5	30	1[illegible]0	[illegible]	740	620	13.4	5.1	950	850
[illegible]	20	17[illegible]	6[illegible]	685	760	1[illegible].4	6.1	1,350[1]	1,[illegible]00
8	[illegible]4	1[illegible]9	68	800	840	13.7	6.1	1,200	1,200[1]

[1] W extrapolated from heart rate 150 beats/min

Blood pressures were recorded with an Elema differential transformer transducer EMT 490 A on an Elema Klinik recorder. Mean pressures were obtained after electrical integration (time constant 0.8 sec). The reference point was taken as the mid thoracic level in the supine position and the articulation of the fourth rib with the sternum in the sitting position (Levegård et al. 1960). During exercise in the sitting position the subject was kept in a relatively constant position by breathing through a fixed mouthpiece to keep the reference level as constant as possible. The cardiac output was measured with the direct Fick method. The oxygen uptake was measured with the Douglas bag technique. Expired air was collected at rest for 8 min and during exercise for 2—3 min according to the extent of the ventilation. Arterial and mixed venous blood was sampled simultaneously during the air-collection for a period of one minute.

Gas volumes were measured with a spirometer and gas samples were analysed for oxygen and carbon dioxide with the Haldane technique (Haldane and Priestly 1935).

Blood gas analyses. Oxygen saturation and hemoglobin concentration were measured spectrophotometrically (Holmgren and Pernow 1958). The oxygen-content was calculated from the oxygen saturation and hemoglobin concentration (Sunderman et al. 1953), the factor 1.34 being used for the oxygen-combining power of hemoglobin. A correction for physically dissolved oxygen was made according to Peters and van Slyke (1932). The reproducibility of cardiac output and stroke volume determination during exercise with the method described was studied by Holmgren and Pernow (1960) and was found to be 5.2 per cent for the cardiac output and 6.8 per cent for the stroke volume.

pH was measured with a micro-Astrup-standard equipment (Radiometer, Copenhagen, Denmark) (Andersen et al. 1960).

P_{CO_2} was determined with the Astrup technique (Andersen et al. 1960) with a reproducibility of 2 per cent. Owing to the systematic difference between P_{CO_2} determined with the Astrup technique and with a P_{CO_2} electrode (according to Severinghaus) 3.0 mm Hg was added to the Astrup-values.

Stand. bicarbonate was calculated according to Andersen and Engel (1960). All measurements of pH were performed immediately after withdrawal and corrected for differences between body temperature and electrode temperature (Bradley, Stupfel and Severinghaus 1956, Holmgren and McIlroy 1963).

The *mechanical efficiency* was calculated from the equation

$$M = \frac{(\text{rate of work, kpm/min}) \cdot 427 \cdot 100}{(\text{oxygen uptake exercise} - \text{oxygen uptake rest}) \cdot 4.9}$$

where $\frac{1}{427}$ and 4.9 are the factors converting kpm and l oxygen to calories.

Lactic acid concentration was determined according to Barker and Summerson (1941) and Ström (1949).

Procedure

Prior to the experiments the volonteers were subjected to a p lot test which ncluded work with successively increasing loads up to a heart rate of 140—150 beats per min The subjects then continued to wo k at this load for 1 hr

On the day of the experiment the subjects arrived at the hospital early in the morning All had had a light meal about one hour before the experimental procedure as started No sedation or qu nidin was given When the cath ters had been introduced cardiac output blood volume blood gas tensions pH standard bicarbonate and pressures were measured in the supine position After this the subject walked over to a bicycle ergometer and started to work immed ately at the selected work load No measurements we e attempted at rest in the s tting posit on This was on account of earlier experience of the tendency of young men to syncope during sim lar experimental condit ons (Bevegård *et al* 1960) The work started immediately at the load that was kno vn to increase the hea t rate after 6 min work to 140—150 beats min Every 10 min for 1 hr expired a r was collected for 2—3 min while blood samples we e with drawn f om the b achial and pulmonary arteries for the determination of oxygen saturation and content carbon-diox de tens on pH standard bicarbonate and lactate Afte 10 and 40—50 min wo k I labelled albumin was injected for blood volume determ nation Rectal temperature ECG heart te brach al arterial pulmonary arterial and right ventricular p essure we e monitored contu uously The work test was continued for 50—60 min and was then stopped and the catheters withdrawn No complicat ons whatsoever occurred before during or after the experiment In two cases the p ocedure was stopped after 50 min work because of falling blood p essures or fatigue The volume of blood thdrawn was of the order of 150—175 ml The fluid losses blood and sweat were compensat d for by continuous saline administration to keep the body we ght constant

Results

An hropometric data are given in Table I

The *total amount of hemoglobin* (Thb) was on an average 812 g (range 680—900 g) corresponding to 11 76 g per kg b w (range 10 84—12 71) which is of the same order of magnitude as that reported in corresponding materials from this laboratory (Holmgren *et al* 1960 Bevegård *et al* 1960) The mean value for the hemoglobin concentration was 13 50 g per 100 ml blood (range 12 4—14 7) which is of the same order of magnitude as that reported in the above materials One subject had hemoglobin concentration below 13 g per 100 ml which is somewhat lo and might be explained by the fact that the subjects were blood donors

The *total blood volume* determined with the alveolar CO method was on an average 6 02 (range 5 1—6 8) or 87 2 ml (range 81 9—92 4) per kg body weight, which is of the same order of magnitude as that found by Holmgr n *et al* (1960) When corrected for body hematocrit variat on (factor 0 91 the mean CO blood volume amounts to 6 61 l

The *blood volume* determined with I tagged albumin was on an average 6 03 (range 5 2—6 7 After 10 m n work the blood volume had decreased to an a erage of 5 60 l a decrease of 4 0 ml After 50 min work the average blood volume was 5 47 l which is not significantly different from the value after 10 min work The corresponding *plasma vol mes* w 3 73 l at rest 3 32 l after 10 min work and 3 20 l after 50 min work

The *heart volume* n the prone position a eraged 775 ml range 685—930 which is somewhat la g r than the value pred cted from THb

The *amount of* *rk* that could be performed at a heart rate of 170 beats min (W) (Table I and II was on an average 1200 kpm min (range 950—1400) There as no

TABLE II. Data obtained at rest and during exercise with heart catheterization in six healthy

Case no	Work load kpm/min	Time min	Pulse rate beat/min	$\dot V_{O_2}$ ml STPD/min	Mechanical efficiency %	$\dot V_E$ BTPS l/min	AVD ml/l	Q l/min	SV ml	Lactic acid mEq/l
2	rest	—	71	3[illegible]0	—	9.3	3[illegible]	10.7	154	—
	900	10	145	2,4[illegible]0	20.6	60.9	114	21.7	150	3.15
	900	20	154	2,4[illegible]0	20.4	65.5	120	20.8	135	3.20
	900	30	163	2,5[illegible]0	19.6	67.3	117	21.9	13[illegible]	3.65
	900	40	166	2,6[illegible]0	18.9	71.3	122	21.8	131	3.35
	900	50	[illegible]	2,6[illegible]0	18.8	[illegible]0.9	122	21.8	128	3.25
	900	60	[illegible]	2,620	18.7	[illegible]	128	21.0	119	2.85
3	rest	—	[illegible]	[illegible]80	—	[illegible].1	44	6.3	83	1.40
	900	10	149	[illegible]	[illegible]	61.4	135	16.8	113	3.95
	900	20	157	[illegible]90	21.2	61.[illegible]	136	16.9	108	—
	900	30	160	2,3[illegible]0	[illegible]1.0	61.8	14[illegible]	16.3	10[illegible]	3.9
	900	40	16[illegible]	2,400	20.3	65.0	148	16.2	[illegible]9	3.65
	900	50	166	2,420	[illegible]0.1	63.1	153	15.8	[illegible]5	3.[illegible]
4	rest	—	8[illegible]	3[illegible]0	—	9.6	4[illegible]	8.2	106	0.70
	900	10	152	2,150	23.9	55.8	120	17.[illegible]	118	2.35
	900	20	161	2,160	23.8	61.9	123	17.[illegible]	109	2.1
	900	30	165	2,240	2[illegible]	68.7	133	16.9	10[illegible]	2.15
	900	40	167	2,280	[illegible]	72.0	137	16.7	100	1.80
	900	50	169	2,300	22.0	75.0	143	16.1	95	1.40
5	rest	—	65	250	—	7.6	43	5.8	88	0.55
	650	10	150	1,[illegible]60	20.5	37.6	118	14.9	99	1.65
	650	20	157	1,800	20.1	40.7	124	14.5	9[illegible]	1.40
	650	30	163	1,830	19.6	45.[illegible]	12[illegible]	15.0	92	1.35
	650	40	1[illegible]0	1,8[illegible]0	19.[illegible]	44.[illegible]	125	14.9	86	1.15
	650	50	1[illegible]8	1,8[illegible]0	19.[illegible]	44.1	118	15.9	89	0.85
7	rest	—	64	330	—	10.6	43	7.6	119	0.85
	1,200	10	144	2,570	[illegible]3	68.8	138	18.3	127	[illegible]85
	1,200	20	153	2,640	4[illegible]	76.2	135	19	128	3.15
	1,200	30	160	2,[illegible]10	24.5	75.[illegible]	143	18.7	117	3.15
	1,200	40	166	2,[illegible]90	23.3	82.9	142	1[illegible].6	116	[illegible]85
	1,200	50	[illegible]	[illegible]	[illegible]3.6	84.5	13[illegible]	20.1	11[illegible]	2.80
	1,200	60	[illegible]8	2,770	23.5	8[illegible].7	141	19.7	111	[illegible]0
8	rest	—	69	[illegible]30	—	6.4	37	6.2	89	1.30
	1,000	10	148	2,290	23.2	53.6	117	19.5	132	2.20
	1,000	20	157	2,[illegible]80	22.2	54.5	127	18.7	119	1.[illegible]0
	1,000	30	161	2,350	22.2	56.0	116	20.6	128	1.45
	1,000	40	166	2,4[illegible]0	21.7	56.9	127	19.2	116	1.9
	1,000	50	169	2,480	21.2	58.5	130	19.1	113	1.75
	1,000	60	172	2,570	20.9	58.5	133	18.9	110	1.[illegible]5

young men

Pressure mm Hg										
RV		PA			PCV	Br A			Blood vol	Plasma vol
S	D	S	D	M	M	S	D	M	l	l
27	11	25	15	—	—	121	71	83	6.5	[illegible]
38	7	29	9	17	13	160	82	104	5 9	3 6
40	5	29	7	18	—	134	74	95	—	—
39	7	34	11	18	—	133	74	93	—	—
42	6	31	5	15	—	144	80	100	—	—
40	6	29	7	15	—	137	74	93	6 0	3 7
—	—	—	—	17	12	133	76	96	—	—
27	13	24	15	20	11	122	87	95	5 6	3 4
42	3	31	15	22	—	188	102	128	—	—
41	2	34	10	20	—	176	93	119	5 4	3 0
42	−1	32	9	19	—	173	95	120		—
40	−4	—	—	—	—	172	98	117	—	—
45	−3	33	9	17	—	154	81	104	5 0	2 7
24	7	19	9	13	8	105	63	77	5 9	3 6
43	1	34	8	16	—	141	71	95	—	—
45	0 5	—	—	16	—	136	71	93	5.2	3 1
42	0	35	11	17	—	117	58	80	—	—
44	−1	—	—	15	—	117	60	79	5 6	3.3
39	−1	34	8	16	—	117	61	76	—	—
23	7	22	11	17	13	132	82	103	5.2	3.2
40	1	27	9	17	—	181	78	105	—	—
35	−1	28	9	16	—	174	79	105	5 9	2 9
34	−0.5	26	6	15	—	165	74	103	—	—
36	0	28	9	16	—	165	80	10	4	2 8
36	0	27	3	15	—	—	—	—	—	—
26	9	21	10	16	10	111	63	83	6 3	3 9
40	15	34	16	22	—	166	2	96	—	—
39	−1	33	13	20	—	152	65	93	6.2	3 7
37	−5	31	12	19	—	142	60	90	—	—
41	−2	29	15	20	—	143	6	91	5 5	3.2
39	−1	35	1	2	—	13	60	90	—	—
40	−1	36	14	22	—	13	6	83	—	—
21	7	17	9	12	9	109	60	77	6 7	4.2
42	[illegible]	40	18	2	—	171	74	100	—	—
43	8	33	19	23	—	165	4	100	6 0	3 6
44	4	31	1	3	—	—	—	93	—	—
47	3	30	18	23	—	154	71	93	—	—
37	−1	30	1	21	—	162	74	103	6 0	3.5
33	−2	31	17	[illegible]	—	154	77	101	—	—

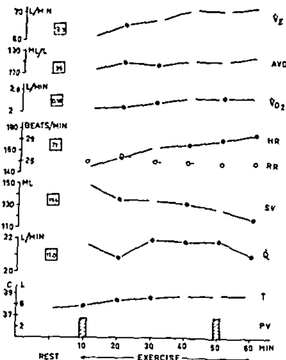


Fig. 1. Hemodynamic and respiratory data (subject no. 2) at rest, data in squares, and during exercise for one hour at a work load of 900 kpm/min on a bicycle-ergometer in the sitting position. $\dot{V}_E$ = total ventilation l BTPS/min. AVD = arterio-venous oxygen difference ml/l. $\dot{V}_{O_2}$ = oxygen uptake ml STPD/min. HR = heart rate beats per min. RR = respiratory rate breaths per min. SV = stroke volume ml. $\dot{Q}$ = cardiac output l/min. T = rectal temperature °C and PV = plasma volume l.

significant difference between W_1 as determined in the sitting or the supine position.

The *heart rate* during the *preliminary* work test measured in the 6 subjects who worked for 50—60 min increased from an average of 154 beats/min (range 143—162) after 10 min work to 174 beats per min (range 165—182). During the experiment including heart catheterization the corresponding figures were 148 beats per min (range 144—152) and 171 beats per min (range 166—178). This is in general agreement with the observations of Bevegård *et al.* (1960) in similar materials that the heart rate response during exercise is not significantly altered by the introduction of the heart catheter.

To summarize, the anthropometric data presented in Table I indicate that the material can be considered as a moderately trained healthy group of young men and that the influence of the experimental procedure is probably unimportant.

Circulatory response during long term non steady state exercise

The pertinent data are presented in Table II, Fig. 1 and 3.

The *oxygen uptake* ($\dot{V}_{O_2}$) at rest in the supine position averaged + 22.4 per cent (range + 5.3 — + 36.3) of that predicted (Harris and Benedict 1919). During exercise $\dot{V}_{O_2}$ after 10 min work corresponded to a mechanical efficiency of *22.7* per cent (range 20.5—26.3). During the prolonged work there was a slow decrease to an average of 20.8 per cent (range 18.8—23.6) after 50 min work, which decrease was significant.

The *heart rate at rest* was on an average 71 beats per min (range 64—78), which is of the same order of magnitude as that in corresponding earlier studies (Holmgren

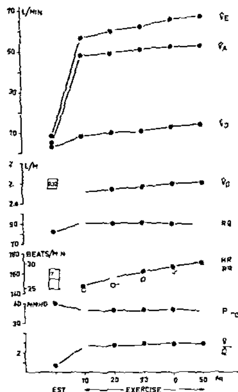


Fig 2 Respiratory data at rest and during exercise in 6 subjects $\dot{V}_E$ = total ventilation 1 BTPS min $\dot{V}_A$ = alveolar ventilation 1 BTPS/min $\dot{V}_D$ = dead space ventilation 1 BTPS/min $\dot{V}_O$ = oxygen uptake 1 STPD/min RQ = respiratory quotient HR = heart rate beats/min RR = respiratory rate breaths/min P_{aCO_2} = arterial carbon-dioxide tension mm Hg $\dot{V}_A/\dot{Q}_c$ = ventilation perfusion relationship

et al 1960) During exercise it rose to an average of 148 beats per min (range 144—152) after 10 min work The continued work caused a slow continuous rise in all subjects *After 50 min work the average heart rate in 6 subjects was 171 beats per min* (range 166—178) In cases 2 7 and 8 who were able to continue for a whole hour the heart rate continued to rise further averaging 175 beats per min (range 172—178)

The *oxygen saturation of arterial blood* was normal at rest and during the whole work period in all subjects

The *cardiac output at rest was 7 5 l min range 5 8—10 7)* which is of the same order of magnitude as that earlier reported (Holmgren *et al* 1960 Bevegård *et al* 1960) During exercise the cardiac output increased after 10 min to values which agreed well with those predicted from the normal regression on $\dot{V}_O$ (Bevegård *et al* 1960) In case 2 the cardiac output remained constant throughout the whole work period If the reproducibility of the cardiac output determination during exercise is taken to be of the order of 5 per cent (Holmgren and Pernow 1960 and using confidence limits of ± 95 per cent the variations during the prolonged exercise period in cases 2 and 8 lie within the variation to be expected from the methodological errors In case 3 there is a significant decrease and in cases 5 and 7 almost significant increases. The magnitude of the changes does not exceed 1 1 min

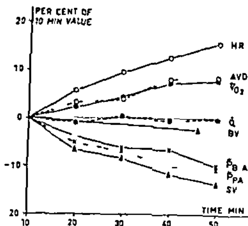


Fig. 3. Hemodynamic responses during long term non steady state exercise in six subjects. $\bar{P}_{BA}$ = brachial arterial mean pressure. $\bar{P}_{PA}$ = pulmonary arterial mean pressure. The other symbols are the same as in Fig. 1. Data are presented as a percentage of the values obtained after 10 min work.

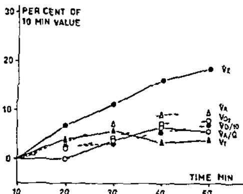


Fig. 4. Respiratory responses during long term exercise. Symbols as in Fig. 3. Data given in per cent of 10 min value.

The *stroke volume* at rest in supine was on an average 106.5 ml (range 83—154). After 10 min exercise the volume had increased in 5 subjects and remained essentially unchanged in case 2. In all subjects the stroke volume decreased successively during the work period from an average of 123.2 ml after 10 min work (range 99—150) to 106.2 ml (range 89—128), or 13.8 per cent of the 10 min value after 50 min work. The 10 min value fell within the normal range of variation for the relationship between stroke volume during exercise in the supine position and THb, heart volume and W sitting except in case 3, who had a stroke volume larger than that predicted from the heart volume (Holmgren *et al.* 1960, Bevegård *et al.* 1960). The finding that the stroke volume during work in the sitting position fell within the normal regression between stroke volume in the supine position and the above mentioned parameters may indicate that only minor orthostatic blood shifts had taken place in this early part of the work period (Bevegård *et al.* 1960).

Intracardiac and intravascular pressures. The technique used allowed the recording of *pulmonary wedge pressures* at rest in the supine position in all the subjects except case 2. The wedge pressure was on an average 10.2 mm Hg (range 8—13). Right ventricular

pressure was recorded both at rest and during work as were also the pulmonary and brachial arterial pressures.

The *systolic right ventricular pressure* was at rest on an average 24.7 mm Hg (range 21—27) and rose during exercise to an average of 40.8 mm Hg (range 38—43) after 10 min work. The mean systolic pressure in six subjects after 50 min work was 39.3 mm Hg. The *end diastolic pressure* in the right ventricle at rest was on an average 9.0 mm Hg and fell after 10 min work to an average of 2.6 mm Hg and after 50 min work to ± 0 mm Hg.

The average *systolic pulmonary arterial pressure* was at rest 21.3 mm Hg (range 17—25) rising after 10 min work to 32.5 mm Hg (range 27—40) and after 50 min work to 31.3 mm Hg (range 27—35). The corresponding figures for *diastolic pressure* were 11.5 (range 9—15) at rest, 12.5 (range 8—18) after 10 min work and 9.5 mm Hg after 50 min work (range 3—15). The *pulse pressure* in the lesser circulation thus increased slightly during the work period. The average *mean pressure* in the pulmonary artery was 15.6 mm Hg (range 12—20), 19.8 mm Hg (range 16—25) after 10 min work and 17.7 mm Hg (range 15—22) after 50 min.

The *systolic brachial pressure* was on an average 116.7 mm Hg (range 105—132) at rest, 167.8 mm Hg (range 141—188) after 10 min work and 145.3 mm Hg (range 117—165) after 50 min work. The corresponding figures for *diastolic pressure* were 70.8 mm Hg (range 60—87), 79.8 mm Hg (range 71—102) after 10 min work and 71.7 mm Hg (range 60—81) after 50 min. The average *mean pressure* was at rest 88.2 mm Hg (range 77—108), 104.7 mm Hg (range 95—128) after 10 min work and 94.2 mm Hg (range 76—104) after 50 min. The *pulse pressure* thus increased from 46 mm Hg at rest to 88 mm Hg after 10 min work and then decreased to 74 mm Hg after 50 min work. With a constant cardiac output the decrease in the systemic arterial mean pressure indicates a decrease in the systemic vascular resistance.

The lactic acid concentration in arterial blood at rest averaged 0.96 meq/l. During exercise it rose to an average value of 2.69 meq/l after 10 min work and then decreased slightly to 2.28 meq/l.

Respiratory response during long term non steady state exercise

The results of the respiratory studies are summarized in Fig. 2 and 4 and the pertinent data given in Table III.

The *minute ventilation* (V_E) increased during exercise to an average of 56.3 l BTPS/min (range 37.6—68.8) after 10 min work corresponding to a *ventilatory equivalent* (V_E/V_{O_2}) of [illegible].0 l BTPS l STPD (range 21.3—27.3) and an *alveolar ventilation* (V_A) of 47.9 l BTPS (range 32.7—60.5). During the continuation of the work V_E rose continuously to an average of 66.8 l BTPS (range 44.1—84.5) after 50 min while at the same time V_A increased only slightly (Fig. 2 and 4).

The increase in total ventilation was effectuated by an increase in both respiratory rate and tidal volume but the changes in rate could not explain the increased dead space ventilation alone.

The *respiratory quotient* RQ at rest averaged 0.8[illegible] (range 0.77—0.90). After 10 min work RQ had risen to 0.91 (range 0.85—0.96) and remained then approximately constant 0.[illegible] during the rest of the work indicating that none of the subjects was fasting.

The *arterial carbon dioxide tension* P_{aCO_2}, *pH and standard bicarbonate* P_{aCO_2} at rest averaged 40.0 mm Hg (range 39—41), pH 7.41 (range 7.39—7.45) and standard

TABLE III Respiratory data at rest and during long term exercice in six healthy young men

Case no	Work load kpm/min	Time min	Resp rate	$\dot{V}_E$ l BTPS/min	V_T ml BTPS	$\dot{V}_O$ ml STPD l min	RQ
2	rest	—	—	9 3	—	380	0 81
	900	10	25	60 9	2 440	2 470	0 94
	900	20	26	65 5	2 570	2 490	0 92
	900	30	25	67 3	2 690	2 570	1 00
	900	40	25	71 3	2 850	2 650	1 00
	900	50	25	70 9	2 840	2 670	1 00
	900	60	25	72 7	2 910	2 680	0 99
3	rest	—	15	7 1	470	280	0 77
	900	10	25	61 4	2 460	2 270	0 91
	900	20	26	61 2	2 350	2 300	0 90
	900	30	23	61 8	2 690	2 390	0 90
	900	40	27	65 0	2 410	2 400	0 89
	900	50	28	68 1	2 430	2 490	0 89
4	rest	—	16	9 6	600	350	0 78
	900	10	30	55 8	1 860	2 150	0 90
	900	20	32	61 9	1 930	2 160	0 92
	900	30	35	68 7	1 960	2 240	0 90
	900	40	44	72 0	1 640	2 280	0 89
	900	50	48	75 0	1 560	2 300	0 87
5	rest	—	18	7 6	420	250	0 87
	650	10	22	37 6	1 710	1 760	0 85
	650	20	17	40 7	2 400	1 800	0 86
	650	30	24	45 2	1 880	1 830	0 86
	650	40	22	44 2	2 010	1 870	0 85
	650	50	22	44 1	2 010	1 870	0 81
7	rest	—	15	10 6	710	330	0 90
	1 200	10	26	68 8	2 650	2 520	0 96
	1 200	20	31	76 2	2 460	2 640	0 93
	1 200	30	28	75 2	2 690	2 670	0 93
	1 200	40	30	82 9	2 760	2 790	0 93
	1 200	50	33	84 5	2 560	2 770	0 92
	1 200	60	36	87 7	2 440	2 770	0 91
8	rest	—	16	6 4	400	230	0 77
	1 000	10	21	53 6	2 550	2 290	0 87
	1 000	20	22	54 5	2 480	2 380	0 86
	1 000	30	22	56 0	2 550	2 390	0 84
	1 000	40	23	56 9	2 470	2 430	0 86
	1 000	50	21	58 5	2 780	2 480	0 84
	1 000	60	20	58 5	2 930	2 570	0 83

RQ = respiratory quotient = $\frac{F_{A_{CO_2}} - F_{E_{CO}}}{F_{A_{CO_2}} - F_{I_{CO}}}$ i e $\frac{V_D}{V_T}$ when V_D includes the

$\dot V_D$ ml BTPS	V_D/V_T	V_D (BTPS) min	Rectal temp C	Arterial pH units	Arterial P_{CO_2} mm Hg	Stand bicarb meq/l	$\dot V_A$ (BTPS) min	$\dot V_A/\dot Q_C$
—	—	2 5	37 6	7 39	39	21	6 8	0 6
—	—	—	37 8	—	—	—	—	—
413	0 16	11 8	38 3	[illegible]36	37	19	53 7	2 [illegible]
1 [illegible]	0 07	5 4	38 5	7 35	3[illegible]	18	61 9	[illegible] 8
274	0 10	7 8	38 7	7 3[illegible]	36	19	63 5	2 9
159	0 06	5 0	38 8	7 36	3[illegible]	18	65 9	[illegible] 0
163	0 06	5 1	38 8	7 36	34	18	67 6	3 2
12[illegible]	0 27	2 5	37 0	7 42	40	24	4 6	0 7
[illegible]04	0 12	8 6	37 5	7 38	34	19	5[illegible] 8	3 1
336	0 14	9 8	37	7 3[illegible]	3[illegible]	19	51 4	3 0
390	0 15	9 9	38 1	7 35	3[illegible]	18	51 9	3 2
46[illegible]	0 19	13 6	38 4	7 35	36	18	[illegible]1 4	3 2
543	0 22	16 4	38 6	7 36	36	19	51	3 3
206	0 34	3 9	37 3	7 3[illegible]	41	[illegible]2	5 7	0 7
[illegible]20	0 12	7 8	37 7	7 37	3[illegible]	19	48 0	[illegible] 7
405	0 21	14 3	38 0	7 37	36	19	47 6	[illegible] 7
451	0 [illegible]3	17 2	38 2	7 39	34	19	[illegible]1 5	3 0
474	0 29	20 9	3[illegible]4	7 39	3[illegible]	20	51 1	3 1
[illegible]07	0 32	26 3	38 6	7 40	36	[illegible]0	48 7	3 0
167	0 40	3 7	36 8	7 41	41	24	3 9	0 7
182	0 11	4 9	37 1	7 3[illegible]	40	[illegible]1	32 7	2 2
[illegible]9[illegible]	0 1[illegible]	5 7	37 7	7 41	38	21	3[illegible] 0	2 4
293	0 16	8 1	38 2	7 40	37	[illegible]1	37 1	2 5
282	0 14	7 1	38.5	7 40	37	21	37 1	2 5
361	0 18	8 8	38 8	7 41	37	[illegible]1	35 3	[illegible]
[illegible] 9	0 3[illegible]	4 0	36 8	7 41	39	23	6 6	0 9
278	0 10	8 3	37 4	7 38	3	20	60 5	3 3
3[illegible]8	0 15	13 0	37 9	7 38	34	19	63 2	3 2
497	0 19	15 0	38 2	39	36	[illegible]0	60 2	3 [illegible]
568	0 [illegible]1	18 3	8 8	—	3[illegible]		6[illegible] [illegible]	3 3
498	0 19	1 8	38 7	[illegible] 40	33	19	66 7	3 3
[illegible]4	0 22	21 0	38 8	7 40	33	19	66	3 4
12[illegible]	0 31	2[illegible]	3[illegible] 4	4[illegible]	40	[illegible]	3 8	0 6
343	0 13	8 1	36 9	4[illegible]	38	3	4[illegible]	[illegible] 3
3 [illegible]	0 14	8	3 3	43	39	23	4[illegible] 8	2 4
4[illegible]	0 18	11	37 7	7 44	39	24	44 8	[illegible]
45[illegible]	0 18	11 4	38 0	7 43	40	[illegible]3	4[illegible]	4
517	0 19	11	38 1	7 44	39	4	46 8	[illegible]
345	0 13	11 7	38 3	7 44	39	[illegible]3	46 8	[illegible]

Valve dead space

bicarbonate 23 meq/l (range 21—26) After 10 min work [H] had increased and pH averaged 7 38 (range 7 37—7 42) This increase was a result of the addition of fixed acids (standard bicarbonate 20 meq/l) in spite of a slight alveolar hyperventilation P_{CO_2} 36 4 mm Hg (range 34—40) During the continued work there was no further increase of fixed acids standard bicarbonate = 20 meq/l P_{CO_2} remained constant at 36 0 mm Hg (range 33—39) and pH increased slightly The acid base status during the work period should thus be characterized as compensated metabolic acidosis

Rectal temperature The rectal temperature at rest averaged 37 0 (range 36 4—37 6) During the work period the temperature rose slowly reaching 38 6 C after 50 min (range 38 1—38 8)

Ventilation perfusion relationship V_A/Q_c The overall V_A/Q_c at rest averaged 0 70 (range 0 6—0 9) After 10 min work V_A/Q_c had risen to 2 72 (range 2 2—3 3) During the continued work it rose slightly to 2 88 (range 2 2—3 3)

Discussion

The experiments were designed to produce a situation in which the subjects could not maintain a steady state of circulation and respiration The changes were planned to take place slowly to allow repeated measurements within one hour The rate of change judged by the change in heart rate (6 beats/10 min) indicates that the aim was reasonably well realized The situation studied can thus be characterized as prolonged non-steady state work

It is reasonable to ask whether the introduction of a heart catheter can have influenced the hemodynamic responses Holmgren *et al* 1960 and Bevegård *et al* 1960 found no effect of catherization when studying the hemodynamic reaction during short periods of exercise (10 min) or when comparing the heart rates during a preliminary work test without catheter or during the work test in connection with heart catheterization The same method for the evaluation of the influence of procedure was used in the present study and no significant difference was observed between the pulse response during prolonged exercise without and with heart catheterization

The use of the direct Fick method for the determination of the cardiac output requires a steady state of oxygen uptake and a v oxygen difference to give valid data Visscher and Johnson (1953) estimating the magnitude of the error induced by a non steady state found that they are serious only when large changes take place as for example during the transition from rest to work In the present experimental set up the changes occurred slowly and probably did not influence the measurements significantly

Circulatory responses (Fig 3) In accordance with the design of the experiments the heart rate rose steadily during the exercise period with a slope of the order of 50 beats per 50 min At the same time the oxygen uptake rose on an average 10 per cent an increase which is probably explained by the fact that at the relative work loads used the subjects tend to move to and from on the ergometer as the time passes The cardiac output remained on an average constant indicating that the stroke volume fell and the a v oxygen difference increased (cf Cobb and Johnson 1963) There was a continuous decrease in the systemic arterial mean pressure in accordance with the observations of Holmgren (1956) and a slight decrease in the pulmonary arterial mean pressure These pressure changes indicate shifts in the blood vessel volume relationship or in the vascular resistances

The primary change in the central hemodynamics during prolonged non steady state exercise in the sitting position is probably a decrease of the stroke volume which is compensated for by an increase in the heart rate to keep cardiac output constant Since the dimensions of the heart remain unchanged the decrease in stroke volume can be explained in the light of defective filling and/or emptying of the heart The filling of the ventricles is determined by the time available for filling and the amount of energy potential and kinetic present in the capacitance vessels in front of the heart The filling energy has a static component (lateral pressure times volume of capacitance vessels) and a kinetic component Changes in the distribution of the blood within the capacitance vessels caused for instance by gravitation will affect the static component and result in a decrease of the stroke volume Such changes take place when rising but are more or less corrected for when exercise is initiated in the upright position with the muscle pump In the present experimental situation such a decrease of the central blood pool might be caused by i) gravitational shifts due to a change in tone of the capacitance vessels ii) decrease in the total blood volume due to increased filtration pressure in the capillaries or iii) shifts due to vasodilatation caused by a heat regulating mechanism In the present study the cause is probably a change in the distribution of the central blood volume since the total blood volume remained unchanged during exercise

Holmgren (1956) Bevegård (1963) found that mechanical systole of the ventricles varied as an approximately linear function of the heart rate The slope of the decrease was such that a change in heart rate from 140 beats per min to 170 beats per min was accompanied by a decrease in the mechanical systole from 24 csec to 20 csec At the same time the mechanical diastole i e the time available to fill the heart is shortened from 21 csec to 16 7 csec (Bevegard 1963) A decrease in the stroke volume due to shifts in the blood distribution would thus probably be further enhanced by an increase in the heart rate of the same order of magnitude in the present experimental situation

Both these mechanisms will affect the filling of the heart. It must be stressed however that we know but little about factors which may affect the contractility and thus the emptying of the heart since we have at present no valid methods to demonstrate a decrease in myocardial contractility

The *respiratory response* (Fig 6) is characterized by a successively increasing minute ventilation effectuated by both an increasing respiratory rate and tidal volume The increased $\dot{V}_E$ does not change $\dot{V}_A$ to any major degree but causes mainly an increase in the dead-space ventilation as indicated by the minor changes in P_{CO_2} The increase in $\dot{V}_D$ is effectuated both by an increase in the respiratory rate and an increase in V_D and probably indicates a change in the regional $\dot{V}_A$ $\dot{Q}_c$ relationships, although the overall $\dot{V}_A$ $\dot{Q}_c$ changes only slightly Such a change might be expected if the perfusion of the upper parts of the lungs decreased during prolonged exercise causing an increased alveolar dead space (Severinghaus and Stupfel 1957) A decrease of the perfusion of the upper parts of the lungs might be caused by gravitational shifts of blood from the pulmonary vascular bed to the legs and is quite compatible with the falling stroke volume observed This explanation is also favoured by the observations of increased alveolar dead space in the standing position and during gravitational stress (Barr Bjurstedt and Coleridge 1959 Bjurstedt *et al* 1962) and by the observation of increased ventilation during exercise with the same work load after bleeding ten per cent of the total blood volume (Gullbring *et al* 1960)

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Acta physiol scand 1964 62 256—262

From the Department of Physiology University of Göteborg Sweden

The Effect of Graded Stimulation of Efferent Vagal Nerve Fibres on Gastric Motility

By

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Abstract

Martinson J *The effect of graded stimulation of efferent vagal nerve fibres on gastric motility* Acta physiol scand 1964 62 256—262 — The gastric motor responses to efferent vagal stimulation of varying frequency and impulse duration were studied in cats The efferent fibres both excitatory and inhibitory exert their maximum effect on stimulation by an impulse rate of about 10 imp/sec Higher frequencies producing little if any further effect The stimulation thresholds of the excitatory and inhibitory fibres depend only on the strength and duration of stimulation pulses and not on their frequency so that high frequency-stimulation is not equivalent to high intensity stimulation i e stimulation with long-duration pulses regarding its ability to produce gastric motor inhibition The findings argue against the inhibition observed being produced by overflowing transmitter substance from aberrant sympathetic vasomotor nerve fibres running in the vagal supply to the stomach

The autonomic nervous control of smooth muscles and glands is generally considered *to be exerted by a fibre discharge of relatively low frequency For instance resting* sympathetic tone seems to be maintained by a discharge rate of usually at most 1—2 imp/sec and hardly ever exceeding 8—10 imp/sec even on intense excitation of the centres except perhaps for the very short periods when phasic adjustments are induced (For references see Rosenblueth 1950 Uvnäs 1960)

As regards the vagal influence on gastric motility a fairly thorough search of the literature failed to reveal any recent relevant investigations In 1925 Veach found gastric motility to increase with the rate of direct vagal stimulation up to about 12—20 imp/sec When the rate exceeded this limit *or* when the strength of the stimulation pulses was increased the responses tended to become weaker or even to change in direction Veach ascribed these effects to Wedensky inhibition and did not consider the possibility of different types of nerve fibres being involved McSwiney and Wadge (1928) on the other hand concluded from their experiments that the initial tone level of the smooth muscle effectors was the dominant factor in determining whether vagal stimulation

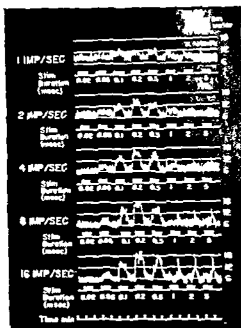


Fig 1 Stimulation of varying frequency and impulse duration Constant pulse intensity 5 volts

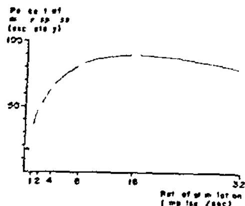
would cause increased or inhibited gastric motility inhibition being found at a high initial tone and *vice versa* Both these views of the functional organisation of the vagal influence on gastric motility seem to have been shared by authors of modern reviews and text books (*e g* Garry 1957 and Nasset 1961)

In a recent investigation of the effect of direct vagal stimulation on gastric motility under experimental conditions allowing exact gradation of pulse duration and strength Martinson and Muren (1963) produced strong evidence of the existence of two types of efferent vagal fibres influencing gastric motility one fibre group being excitatory the other one inhibitory The stimulation threshold of the latter was high and thereby suggested that the inhibitory influence was conducted by thinner fibres Furthermore unlike the effect of the excitation fibres the responses elicited by inhibitory fibres were not abolished by atropine The exact mechanism by which the inhibitory fibres exerted their influence on the gastric smooth muscles was thus obscure

The purpose of the present study was to ascertain whether the stimulation frequency *per se* is of any crucial importance in determining the direction of the gastric motility response under circumstances where the other stimulation characteristics are rigidly controlled It was thought that such a study might help to reveal in greater detail the functional organisation of the vagal control of gastric motility

Material and methods

Twenty cats weighing between 1 5 and 3 kg were used The animals were deprived of food for about 24 hours After initial anesthesia with ether a mixture of chloralose and urethane 0 and 50 mg per kg b w respectively was administered i v A rubberball on capabl [illegible]



Frequency response diagram of excitatory responses to efferent vagal nerve stimulation
(Means of 14 experiments)

about 50—60 ml without distension of its wall was passed down the esophagus into the stomach where it was inflated with only 30—40 ml of air so that the original pressure within the balloon could not contribute to the pressure recorded. The balloon was then withdrawn to the cardia to ensure a constant position, after which it was connected to a water manometer recording on a kymograph. The blood pressure was registered continuously by a mercury manometer connected to one of the femoral arteries.

The vagal nerves were exposed in the region of the neck and divided. The skin of the neck was folded to form a trough for liquid paraffin which was kept at body temperature to prevent drying and cooling of the nerve. The peripheral end of one nerve at a time was placed on bipolar silver electrodes. In some of the experiments the vagal trunks were dissected free below the diaphragm and directly stimulated at that level. The stimulation electrodes were connected to a stimulator delivering square wave impulses (Grass mod. 1 S4).

In order to facilitate statistical treatment of the data a geometric series of frequencies was used, 0.5, 1, 2, 4 ... to 32 imp/sec. For each frequency employed a series of impulse durations was run, the voltage being kept constant throughout the experiment. Drugs were administered i.v. and the doses were for atropine 0.1 mg/kg b.w., carbachol 10.1—0.. mg/kg, histamine 0.1—1 mg/kg.

Results

Fig. 1 illustrates a characteristic response to vagal stimulation at a constant voltage and the effect of stepped increases of the pulse duration at various stimulation frequencies. By gradually increasing the duration of the pulses the approximate stimulation threshold of the excitatory fibres can be assessed from the barely visible elevation of the intragastric pressure. It is seen from the figure that at all the frequencies used this threshold level of pulse duration was the same throughout the series. Only when very low frequencies were used, below 1 imp/sec, was it difficult or impossible to decide with certainty when the tonus was significantly increased, because at such low rates the responses are small even when all the fibres are excited. Further increases in pulse duration above the threshold level produced further increments of the tonus, particularly when higher frequencies were used. However, irrespective of the frequencies used at a certain duration of the pulse, in this experiment 0.5 msec, the excitatory response decreased in height, and on further prolongation of the stimulation pulses the response was more or less completely suppressed. In addition, stimulation with such long pulses was often followed by depression of gastric tone for a fairly long time after the stimulation had been stopped. The experiment illustrated in Fig. 1 is in this very respect an

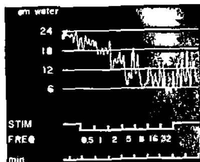


Fig 3 Inhibitory responses after treatment with atropine 0 1 mg/kg and carbachol 0 1 mg/kg Stimulation frequency changed according to scale Stimulation pulses 5 volts 5 msec

exception which might depend on the initially low tone which did not permit further relaxation The after relaxation as a sign of activation of inhibitory vagal nerve fibres was described by Martinson and Muren (1963)

In each experiment variation of the frequency of the impulses was studied for its effect on the response of both stimulatory and inhibitory fibres The strongest excitatory responses were usually recorded at a voltage of 4—5 and a pulse duration of 0 1—0 2 msec In each experiment the strongest response was said to be 100 per cent and the responses obtained at other frequencies but with the same voltage and pulse-duration were expressed as percentages of the strongest response The calculated means of all the responses expressed in such a way are given in Fig 2 It should be noted that the frequency at which the maximal response was recorded varied somewhat It was usually 8 or 16 and occasionally 32 imp/sec This explains why none of the mean values in Fig 2 reached the 100 per cent level The curve nevertheless clearly shows that the strength of the response increases rapidly with the rate of stimulation from 1 to 8 imp/sec at which rate the response is almost maximal Then the curve flattens out and further increases in the rate of stimulation produce no significant further increase of the response

The frequency of stimulation and the strength of the inhibitory responses were then studied for any correlation As long as the low threshold excitatory fibres were free to exert their action the inhibitory influences revealed themselves merely as a more or less pronounced depression of the excitatory responses and their dependence of the stimulation rate could then hardly be determined It can be seen however (Fig 1) that the suppression of the excitatory responses becomes more prominent in the upper frequency range despite the then presumably intense excitatory effects

However since atropine will block the excitatory but not the inhibitory fibre responses (Martinson and Muren 1963) injection of this drug will facilitate demonstration of the relationship if any between the stimulation frequency and strength of the inhibitory effect On the other hand after atropine the tone of the stomach is often so low as to create unfavourable conditions for quantitative estimation of the inhibitory effects because they will all necessarily be small However injection of large doses of carbachol (or sometimes histamine) in addition to the atropine dose often stimulated the activity of the gastric smooth muscles considerably During these conditions the inhibitory responses to high threshold vagal stimulation were correspondingly increased Such an experiment is illustrated in Fig 3 where atropine (0 1 mg/kg) had

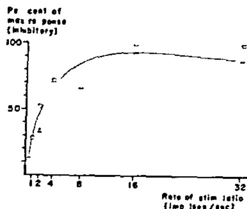


Fig 4 Frequency-response diagram of the inhibitory responses to efferent vagal nerve stimulation. Uninterrupted line calculated means from 8 experiments Squares frequency response diagram with artificially increased initial tone Triangles low initial tone see text.

blocked the excitatory vagal responses while a large dose of carbachol (0 1 mg/kg) had raised the basal activity of the gastric smooth muscles. Vagal stimulation at increasing rates produced decreasing tonus when pulses of 5 msec duration and a voltage of 5 was used It is clear from the tracing that the frequency response relationship was essentially the same as for the excitatory fibres

Since these inhibitory responses were elicited against a background of artificially raised smooth muscle activity it might be objected that the frequency response relationship might have been different if the initial smooth muscle activity had not been artificially increased But this objection can be refuted by the experiment illustrated in Fig 4 in which thre relationship between the frequency and the inhibitory response was estimated in one animal (△) in which the initial smooth muscle activity was not increased (maximal inhibitory response 2 6 cm of water) and in one (□) in which, judging from the intragastric pressure basal tone was increased by carbachol so as to permit a maximal inhibitory response of 15 cm of water

Discussion

The results obtained by Veach (1925) indicated that gastric motility could be inhibited by increasing either the intensity or the frequency of vagal stimulation. He concluded that "The stronger the stimulation, the lower is the frequency required to produce inhibition It should be pointed out in this connection that his study was performed before stimulators for exact grading and characterization of the pulses were available and before it was fully realized that functionally different fibre groups in a nerve trunk often have different stimulation thresholds The results reported by Martinson and Muren (1963) confirm Veach s findings regarding high intensity stimulation insofar as inhibitory responses then become predominant. It should also be mentioned that for reasons evident from the results obtained by Martinson and Muren (1963) in the following discussion the term "high intensity stimulation is used as equivalent to "stimulation with long-duration pulses

The present findings do not confirm Veach s view that an increase in frequency alone is sufficient to produce inhibition even if the intensity is kept low On the contrary

inhibition does not appear until a certain threshold intensity has been passed and it then occurs whether the frequency is low or high These inhibitory effects show a frequency response relationship of the same type as that for the excitatory fibres and all the observations made in the present investigation indicate that they are brought about by a specific set of inhibitory fibres Nevertheless the inhibitory effects of the present study seem to be the same as those noted by Veach at both high intensity and high frequency stimulation It should be recalled that in Veach s study it was hardly possible to grade the stimulation parameters within such wide limits as in the present study and presumably many of the effects recorded by Veach were due to a simultaneous activation of both excitatory and inhibitory fibres

A certain depression of the neurogenic influence will appear in any neuroeffector unit as long as the rate of stimulation is high enough simply because some link in the transmission of the impulse to the effector will ultimately fail if the rates used exceed the capacity of the system in this case it might not be out of place to use the old term Wedensky inhibition (Rosenblueth 1950 pp 188—190) Such a phenomenon is easily brought about also in sympathetic neuroeffectors and Folkow (1952) suggested that it is at least partly due to failure of the most sensitive link in the chain the transmitter release mechanism Such an unspecific type of supression of effector responses however has nothing in common with the specific inhibition dependent on the intensity or duration of the stimulation pulses seen in the present experiments since that inhibition becomes maximal at those rates of discharge bordering what is generally considered to be the upper limit of the normal range of the autonomic nervous system

The present observations argue strongly for two sets of efferent vagal fibres affecting gastric motility one low threshold group of excitatory fibres and one high threshold group of inhibitory fibres The full range of excitatory and inhibitory effects can be brought about by varying the discharge rate between zero and ten impulses per second Since the inhibitory responses described here are not abolished by atropine (cf Martinson and Muren 1963) an adrenergic peripheral mechanism responsible for the inhibition has been assumed This has been evidenced by Greeff Kasperat and Osswald (1962) and by Paton and Vane (1963)

Concerning inhibitory neurogenic effects on intestinal smooth muscles neither activation of bulbar sympathetic centres nor direct splanchnic stimulations appear to involve any activation of specific inhibitory nerve fibres running to the intestinal wall The inhibition then induced appears to depend largely on the blood borne catecholamines from the adrenal medulla and possibly to some degree on overflow of the transmitter released at the vasoconstrictor nerve endings when the sympathetic nerves are stimulated at supraphysiological rates (Celander 1959 and Kock 1959) Since the effects described in the present study are obtained at low frequencies the concept of overflowing transmitter has to be ruled out The short latency argues against mechanisms involving blood borne catecholamines from the adrenal medulla

The physiological significance of the inhibition produced by efferent vagal stimulation is as yet obscure The most well known inhibitory mechanism of the stomach is its remarkable ability as a reservoir organ to adjust its volume and mural tension to its contents In this so-called receptive relaxation (Cannon and Lieb 1911) a vagal inhibition of stomach motility may participate Cannon and Lieb noted that the relaxation was abolished by vagotomy It is also known that sham feeding producing gastric secretion will inhibit gastric motility e g Olbe and Jacobson 1963 This in turn may be compared by the results preliminary reported by Martinson (1[illegible]) that

gastric secretion is elicited by fibres with the same stimulation characteristics as those producing inhibition of motility Until further experimental data have been produced however any discussion of the functional significance of the inhibitory vagal nerve fibres must be speculative

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Collateral Blood Flow in the Myocardium of Dogs Measured with Krypton^{85}

By

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Abstract

Johansson B E. Linder and T Seeman *Collateral blood flow in the myocardium of dogs measured with Krypton* Acta physiol scand 1964 62 263—270 — Methods have recently been developed for determination of regional blood flows from wash-out curves recorded externally after close arterial injection of radioactive Krypton (Kr) In the present study this technique has been applied to measurement of collateral capillary blood flow in the myocardium of dogs during acute occlusion of coronary arteries A monoexponential fall in activity was found during elimination of Krypton from the normal myocardium indicating a homogeneous distribution of blood flow The wash-out curves recorded during coronary occlusions could be dissolved into two separate components There was a rapid phase related to the elimination of Krypton from normally perfused regions of the myocardium and a slow phase due to the desaturation of the ischemic portion The magnitude of the collateral capillary blood flow was determined from the decay rate of the slow component The relative size of the ischemic region could be estimated from the zero time values of the two phases Collateral blood flow seemed to be fairly homogeneously distributed within the ischemic part of the myocardium Release of an occlusion was followed by a rapid fall in activity in association with the reactive hyperemia

Acute occlusion of a coronary artery will produce a relative ischemia in the corresponding myocardial region due to the fact that the myocardium is supplied mainly by end arteries The ischemic area will however receive some nutritional blood flow *via* interarterial communications The survival of the myocardial cells will largely depend upon the flow capacity of this collateral circulation in relation to the actual metabolic needs Clinical and experimental studies have shown the extent of cell necrosis to be less than expected from the vascular anatomy probably due to significant collateral blood flow



Fig 1 Schematic illustration showing the preparation of the anterior descending coronary artery for Krypton administration and occlusion Left retrograde catheter in a small proximal side branch Right fine polyethylene catheter sutured through the arterial wall

Collateral circulation has been studied experimentally mainly by *measurement of* retrograde flow or blood pressure in the artery distal *to an occlusion Neither of these* methods can be considered to provide direct information on the magnitude of the nutritional blood flow in the ischemic region Indicator clearance techniques can at least from the theoretical point of view be expected to measure collateral capillary blood flow more adequately In a comparative study by Levy Imperial and Zieske (1961) the *values for coronary collateral blood flow* calculated from myocardial uptake of I b were significantly higher than those obtained by simultaneous retrograde flow measurements

Methods have recently been developed for determination of regional blood flow by external recording of wash-out curves after close arterial administration of solutions of radioactive inert gas (*e g* Ingvar and Lassen 1962 Herd *et al* 1962 Thorburn *et al* 1963) Myocardial blood flow was measured with this technique in intact dogs with implanted coronary catheters by Herd *et al* (1962) using Krypton When recording externally over the heart they found a monoexponential decay of activity *from which coronary blood flow could be calculated In the present investigation a* similar technique has been used in an attempt to estimate collateral capillary blood flow during acute coronary artery occlusions in open chest dogs

Methods

The experiments were performed on 15 mongrel dogs anesthetized with pentobarbital intravenously and artificially ventilated in an open system after curarization and intubation Left thoracotomy was made and the pericardium was incised and sutured to the thorax wall in order to minimize the changes in the position of the heart due to the ventilatory movements Two or three short sections of the anterior descending coronary artery or of its main branches were dissected and slings of thin polyethylene catheters were placed around them to be used for occlusion A catheter for *intraarterial administration of* and catheter was inserted either into the central end of a small proximal side branch or directly through the wall of the main coronary artery (Fig 1) In the latter case a polyethylene catheter (PE 60) with a side opening he ted and drawn to fit an unclosed atraumatic vascular needle was sutured into the vessel In larger *dogs the catheter was placed* with this technique in a more distal part of the artery without interfering with the normal flow The vessel could then be clamped *above* the site of Kr inject on and collateral pressure could be recorded

Catheters were introduced into the left ventricle and into one of the femoral arteries for pressure recording

A gamma scintillation detector with a 2 inch NaI crystal dwelled 10 cm inside the opening of a lead collimator was pos *tioned above the anterior wall of the left ventricle The circular* collimator opening measured 5 cm in diameter Gamma activity was recorded on a potentio

meter writer (Kipp Micrograph or Varian recorder) coupled to the ratemeter which converted the output of the photomultiplier tube (Nukleonik AB Goteborg) The Micrograph was run at a paper speed of 4 cm/min and the Varian recorder at 1 7 cm min The time constant of the ratemeter was set at 0 3 and 1 sec fo the two recorders respectively

Krypton gas (English Radiochemical Centre) was equilibrated with saline in airtight syringes Of this solution 1—1 5 ml was injected into the coronary artery during 5—10 sec After two or three recordings of normal myocardial wash-out curves the effect of coronary occlusion on the desaturation process was studied The anterior descending artery or one of its branches was instantaneously occluded as soon as the Krypton injection was completed and the decay of activity had begun The times of onset and release of occlusion were marked on the records In some experiments Krypton was administered after clamping the artery proximal to the injection catheter

Left ventricular and arterial blood pressures were continuously recorded with electromanometers (Elema) on a direct writing 4-channel oscillograph (Mingograph Elema) One or two ECG-leads were registered as well

At the end of the experiments Keaton fast green (Ciba) was injected into the coronary artery After exsanguination of the dog the heart was weighed and an attempt was made to estimate the size of the myocardial regions subjected to arterial occlusions

Results

The type of recordings obtained after injection of Kr into the anterior descending coronary artery is illustrated in the upper part of Fig 2 A As shown below the logarithm of the activity values plotted *versus* time gives a practically straight line indicating that the wash-out of Krypton from the myocardium can be looked upon as a monoexponential process The small deviation from the straight line seen at the end of the plotting has been ascribed to extracardiac activity Herd *et al* 1962)

The elimination of an inert gas from a constantly and uniformly perfused tissue can be described by the equation

$$C_t = C_0 \cdot e^{-k}$$

provided that the arterial concentration of the gas is zero or negligibly low during the wash-out period (Kety 19) In this formula C_t and C_0 represent tissue concentrations at time t and 0 respectively The constant k represents $\frac{f}{V \cdot \lambda}$ where f is blood flow per unit time V is tissue volume and λ is the tissue blood partition coefficient of the inert gas At half time of decay ($t_{\frac{1}{2}}$) C_t is by definition $\frac{1}{2}$ C_0 and the following relationship is obtained

$$k \cdot t \;=\; \log$$

Blood flow can then be calculated in ml min 100 g of tissue from the equation

$$F = \frac{0\ 693 \cdot \lambda \cdot 100}{}$$

where t expressed in min and is the spec f weight of the tissu

In the recording of Fig 2 A the activity in cpm is directly proportional to the tissue concentration of Kr and myocardial blood flow can thus be calculated as above The half time of decay is 31 sec which gives a coronary blood flow of 126 ml min 100 g if the specific weight of the myocardium is set at 1 05 and λ assumed to be 1 0 Hansen *et al* 1956 Herd *et al* 1962)

The effect of coronary occlusion on the myocardial desaturation process is illustrated in Fig 2 B Kr was injected into the anterior descending artery as in A but at the arrow on in the original recording a branch of the artery was instantaneously occluded In this case the semilogarithmic plotting gives a decay curve which shows a gradually decreasing steepness until it finally approaches asymptotically a straight line For

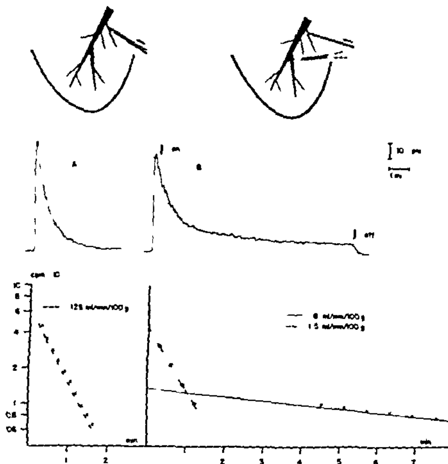


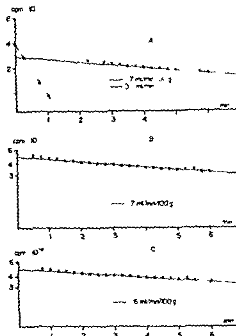
Fig 2 Original recordings and semilogarithmic graphs of wash-out curves after i.a. injection of Kr*
A Monoexponential decay curve during elimination of Kr* from normally perfused myocardium
B Recordings obtained when a main branch of the anterior descending artery was occluded after the injection of Kr*
In the semilog graph the curve is shown to consist of two components corresponding to desaturation of normal and ischemic myocardium respectively

further analysis of the curve this asymptot was extrapolated to time zero The differences between simultaneous ordinate values of the asymptot and the curve were plotted separately in the semilog graph and resulted in a new steeper straight line This analysis of the desaturation curves obtained during coronary artery occlusions indicated that they can be treated as originating from a system of two parallel compartments

The activity (A) recorded during elimination of radioactive inert gas from such a system can be expressed as the sum of two monoexponential processes

$$A = M_1 \; C_0 \; e^{-kt} + M \; C_0 \; e^{-kt}$$

Since the branch of the coronary artery was not occluded until the Kr* injection was completed tissue concentration at time zero (= onset of occlusion) is equal in all regions of the myo-



F g 3 Semilogarithmic representation of re ord ngs obtained during three consecu tive occlusions at the same level of the anterior descend ng coronary artery
A Clamp ng of the artery after p oximal injecti on
B Clamp ng of the artery after distal injec t on.
C Clamp ng of the artery prior to distal injection
The values for collateral blood flow calcu lated from the slo component of A and from B and C are nearly the same

cardium supplied by the main artery and its ramifications M means the mass of tissue which is cleared at rate k and M_s the mass cleared at rate k_s In these M values are included how ev r also fa tors regard ng the geometry of the detector in relation to the t o masses

The rate constants k_1 and k) are obtained from the t values of the two straight lines in the semilog graph of Fig 2 B and the blood flows of the two compartments can be calculated to be 8 and 115 ml/min 100 g respectively The lower value has been interpreted as representing collateral blood flow in the ischemic region and the higher value as related to normally perfused myocardium

The intercepts of the zero time ordinate and the two monoexponential compartment lines in Fig 2 B represent the contributions of each of the myocardial portions to the total activity recorded at the onset of occlusion Since the concentration of the indicator is equal (C_o in both regions at this moment their relative contributions to total ac tivity will depend upon their relative sizes. The quotient of the activity values at the abovementioned intercepts will correspond to M_1 M_2 The size of the ischemic area in relation to the mass of myocardium perfused at normal rate by the patent branches of the anterior descending artery can thus be estimated from this quotient As mentioned above allowance must be made for possible differences in the geometry of th detector with respect to the tw regions Such differences are considered to be small under the actual experimental circumstances due to the wide opening of the collimator and the relatively long distan e from heart to crystal The activit values for the slo and the rapid phase at time zero correspond in Fig 2 B to approximately 13 000 and 45 000 cpm respectively This means that the ischemic region makes up about 20—25 of the myocard al tissue to which Kr was administered in this very experiment.

Release of a coronary artery occlusion was regularly followed by a rapid wash-out of Kr [85] from the previously ischemic region (see Fig 2 B) This final part of the recording can be used for studying the reactive hyperemia in the myocardial vasculature

Fig 3 shows the semilogarithmic representation of three consecutive recordings obtained in an experiment where two different catheters were used for the administration of Krypton With the needle technique described above one catheter had been inserted into the proximal section of the anterior descending artery and another one into the same vessel approximately half way between the apex and the base of the heart The artery was prepared for occlusion between the two catheters Fig 3 A shows the semilog graph of the curve recorded when the coronary artery was clamped after injection of Krypton through the proximal catheter It shows the same general characteristics as the curve of Fig 2 B and it has been analysed in the same way The blood flow values calculated from the rapid and slow component in Fig 3 A are 130 and 7 ml/min/100 g respectively The Krypton solution was then injected *via* the distal catheter and the coronary artery was clamped afterwards at the same level as in A. The recording now obtained is represented in B Except for the initial slightly steeper portion this wash-out curve is a slowly falling monoexponential Its rate of decay is practically the same as that of the slow component in A When the indicator was injected through the distal catheter after occlusion of the artery the curve of Fig 3 C was obtained This curve is very similar to that in B but the fall in activity is somewhat slower The blood flow values calculated from B and C and from the slow component of A do not differ significantly and the three decay curves are therefore considered to represent identical collateral blood flows in the ischemic region Administration of Krypton into the distal part of a clamped artery as in C is possibly less adequate since the fall in arterial concentration of indicator towards zero values might be delayed This could be a source of error responsible for the somewhat lower k value of C as compared to B and to the slow phase of A

The catheter inserted distal to the site of occlusion permits measurement of the collateral pressure With known values for mean arterial blood pressure for mean collateral pressure and for collateral blood flow it is possible to calculate separately the flow resistance (pressure/flow) of the interarterial collaterals and of the peripheral vessels in the ischemic myocardium (Johansson Linder and Seeman to be published)

The magnitude of the control blood flows in the myocardium has been in the range of 55—150 ml/min/100 g in the present experiments Collateral capillary blood flow estimated as described above has varied between 5 and 30 ml/min/100 g in the different animals and under the different experimental conditions The latter values are evidently influenced by the size of the ischemic area and by the general circulatory state of the animal The effects on the collateral flow of changes in arterial blood pressure in heart frequency and in the rheological properties of the blood are subjected to systematic analysis (Johansson, Linder and Seeman, to be published) The values for myocardial blood flow during reactive hyperemia calculated from the wash-out curves have been in the range of 150—300 ml/min/100 g

Discussion

The monoexponential decay of activity recorded over the normal myocardium during the wash-out of Kr [85] indicates that the capillary flow is homogeneously distributed and stable. This is in agreement with the findings of Herd *et al* (1962) Influence on the

myocardial desaturation curves of Kr in the lesser circulation and in the alveoli could be effectively reduced by collimation in the present experiments

The magnitude of the myocardial blood flow has been in the range of 55—150 ml/min/100 g and values of 100—120 ml have been most frequently obtained These blood flows are higher than those found in intact unanesthetized dogs by Herd *et al* (1962) but this can be adequately explained by differences in the circulatory state of the animals

The primary aim of this investigation was to study the effects of coronary artery occlusions on the myocardial desaturation curves in an attempt to measure collateral capillary blood flow The analysis of the recordings obtained after coronary occlusion distal to the site of injection showed that most of the curves could be dissolved into two distinct components of which the slow one has been interpreted as representing flow in the ischemic region. This might be an oversimplification from the theoretical point of view since collateral blood flow cannot *a priori* be expected to be homogeneously distributed (*cf* Cutarelli and Levy 1963) It is therefore probably more correct to consider the slow component as consisting of a family of exponentials with small numerical differences They are evidently too similar to appear as separate components in the desaturation curves of Fig 3 B and C These curves should with the technique used originate exclusively from the ischemic myocardium

The k value of the rapid component has been found to correspond closely to that obtained from preceding recordings without occlusion (*cf* Fig 2 A and 2 B) and can thus be considered to represent flow in normally perfused myocardium Intermediate blood flow rates may exist in the transitional zones between the ischemic region and the normal tissue These zones seem however to be of relatively little mass since the desaturation curves are so markedly dominated by the two major compartments This view is also supported by the observations of usually very distinct borders of the ischemic area after *in vivo* injection of dye The initial slightly steeper portion of the decay curves in Fig 3 B and C might correspond to elimination of Krypton from a small mass of borderline tissue

For the interpretation of the composite wash-out curves it must also be assumed that flows remain constant in the two compartments during the measuring period The arterial pressure distal to the occlusion drops to a low level within 1 or 2 sec and stays practically unchanged thereafter One can further expect that rapidly accumulating metabolites will induce maximal vasodilatation in the ischemic myoardium soon after occlusion A sufficient constancy of flow in the two compartments is therefore likely to prevail if the general circulatory state of the animal is otherwise unchanged

The approximations and assumptions discussed above will hardly invalidate the actual treatment of the wash-out curves The fact that *coronary* occlusion produces a change in a desaturation process which is originally monoexponential makes the present application of compartment analysis less susceptible to theoretical objections The method described is now used for an experimental analysis of the circulatory factors which influence the collateral blood flow of the myocardium during acute coronary artery occlusions The collateral circulation after ligation of a coronary artery could probably be studied in chronic experiments by external recording of wash-out curves after injections of Krypton through a distally implanted catheter

In the estimation of the relative size of the ischemic area from the zero time activities as done in Fig [illegible] B the results were well reproducible in double determinations provided that the time of occlusion was marked off exactly on the recordings A separate series

of experiments has shown that the M_1/M_2-quotients obtained at alternative occlusions of the anterior descending artery or its branches at different levels correlated well with what could be expected from the vascular anatomy (Johansson Linder and Seeman to be published)

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Iron Exchange between Transferrin and the Placenta in the Rat

By

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Abstract

Laurell C B and E Morgan *Iron x hange bet en t ansferrin and the placenta in the r t* Acta physiol scand 1964 6? 271—?79 — The process of iron exchange between transf rrin and the placenta as studied *in vitro* using Fe rat serum or purified at transferrin labell d with I and placental tissue The yolk sac placenta bound mor transferrin and took up iron more rap dly than did the allantoic pl centa Th amount of transferrin bound increased rap dly fo the first 15 minutes but thereafter howed little further increase the amount bound increased as the transferrin concentration was increased but was unaffected by the degree of its saturation with iron Iron uptake increased stead ly during incubation as great r when the iron con centration wa ncreased, but was unaffected by increa ing the transferrin concentrat on with a constant ron concentration The release of iron from placenta was slower than that of trans ferrin and was less nto a transf rr n free solution than into plasma The ntravenous injection of rat transferrin n amounts sufficient to approximately double the plasma concentration d d not alter the subsequent transfer of Fe from maternal plas na to the fetuses The results how that the mechan sm of iron exchange between transferrin and rat placenta s very similar to that between transferrin and ret culocytes

In the non pregnant animal most of the iron passing through the plasma is taken up by erythropoietic tissue The mechanism by which iron is transferred from plasma trans ferrin to immature erythroid cells has been studied in vitro by several workers using purified transferrin and reticulocytes (Jandl *et al* 1959 Schade 1961 Jandl and Katz 1963 Morgan and Laurell 1963) During the later part of pregnancy a relatively large fraction of plasma iron turnover is directed away from maternal erythropoietic tissue and towards the conceptus (Vosburgh and Flexner 1950 Bothwell *et al* 1958 Davies *et al* 1959) In this process iron is transferred from maternal plasma transferrin to the placenta and hence to foetal plasma transferrin (Pommerenke *et al* 1942 Vosburgh and Flexner 1950 Bothwell *et al* 1958) The placenta must th refore have considerable capacity for removing iron from transferrin The present experiments were de-

to study both *in vitro* and *in vivo* some aspects of the mechanism by which iron is exchanged between transferrin and the placenta. The 20 day pregnant rat was chosen as the experimental animal because of the very rapid transfer of iron to the fetus which occurs during the last few days of pregnancy in the rat (Nylander 1953, Morgan 1961 a).

Materials and methods

Rats

Four month old albino rats were mated. The day of insemination was determined by daily examination of vaginal smears for spermatozoa. The experiments were performed on the twentieth day after spermatozoa were observed.

Transferrin

Transferrin was isolated in the iron-saturated form from rat plasma by a previously described procedure (Morgan 1964 a) which involved ammonium sulphate precipitation, DEAE-cellulose chromatography and zone electrophoresis. Iron was removed either by dialysis against EDTA or by gel filtration (Morgan 1963). The purified transferrin in a concentration of 10 gm per 100 ml migrated as a single band on paper electrophoresis. Before use in the present experiments it was compared with fresh rat plasma transferrin for its ability to give up iron to rat reticulocytes (Schade 1961). Only transferrin preparations which behaved satisfactorily according to this test were used. In order to be sure that the results obtained with purified transferrin could be applied to fresh plasma all *in vitro* iron uptake experiments were performed using freshly obtained rat plasma as well as with the isolated transferrin. In no experiments were significant differences found between the two sets of results.

Radioactive isotopes

Iron-59 was obtained from Abbots Ltd., North Chicago, Illinois, in the form of ferrous sulphate solution containing ascorbic acid. The Fe was mixed with carrier iron and bound to transferrin by incubating with transferrin solutions or rat plasma for 30 min at 37° C. Carrier free I was purchased from the Radiochemical Institute, Amersham, England. Iron-saturated transferrin was labelled with this isotope by the iodine monochloride method of McFARLANE 1958 to an average extent of 0.5 to 1.0 iodine atom per protein molecule. The transferrin was used within 3 days of labelling.

Experimental procedure

In order to measure transferrin and iron uptake weighed samples of sliced placental tissue were incubated at 37° C with labelled transferrin or plasma for different time periods and then washed 5 times with cold physiological saline at 4° C. Radioactivity was determined in the washed placenta. Because of known differences in iron content between allantoic and yolk sac placenta of the rat (Nylander 1953) separate experiments were performed using the two types of placenta. In some experiments the iron concentration of the incubating solution in different tubes was maintained constant while the transferrin concentration was varied. In another series of experiments the effect of various enzyme inhibitors on placental iron uptake was determined by incubating the placental tissue with the enzyme inhibitor dissolved in 0.9 per cent sodium chloride — 0.01 M sodium bicarbonate for 15 min, then adding an equal volume of plasma Fe and continuing the incubation for a further 30 min.

In order to measure the release of transferrin and iron from placental tissue the sliced placenta was incubated for 15 min with transferrin I — Fe — or plasma — Fe, washed five times and then reincubated for different time periods in 1 ml volumes of either plasma or sodium chloride — bicarbonate solution. At the end of the reincubation time 10 ml cold physiological saline was added, mixed with the contents of the tube and centrifuged at 4° C. Radioactivity was determined in the supernatant solution and in the placental tissue. In certain experiments the effect of inosine and hypoxanthine and various enzyme inhibitors on iron release from the placenta was studied by dissolving these substances in the reincubation plasma before adding it to the labelled placenta.

In a typical experiment a 20 day pregnant rat was anesthetised with ether, the abdomen and uterus opened and the placentae were removed and immediately placed in ice-cold solution consisting of 0.9 per cent sodium chloride, 0.01 M sodium bicarbonate and 0.2 per cent glucose

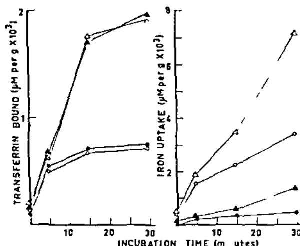


Fig 1 Transferrin and iron uptake by rat allantoic (○ ●) and yolk sac (△ ▲) placenta *in vitro* The iron concentration of the incubation solution was 30 µg per 100 ml (● ▲) or 3?0 µg per 100 ml (○ △) while the TIBC was 320 µg per 100 ml

with the pH adjusted to 7 4 with hydrochloric acid The place itae were quickly washed with this solution dried ith filter paper sl ced by hand us ng a ra or blade and samples were transferred to weighed test tubes which were then rewe ghed and placed in an ice bath The we ght of allantoic placenta in each tube was usually 0 ? to 0 4 g and of yolk sac placenta 0 1 to 0 15 g One ml volumes of icecold transferrin I — F dissolved in the abo e solution or plasma — Fe were added to the tubes One tube from each placenta type was not incubated and wa used to obta n the in t al (zero minute) value for transferrin and or in iron uptake The other tubes were equ librated with a 95 per cent o ygen — 5 per cent carbon d ox de gas mixture stoppered and placed in a shaking water bath at 37 C At measured time ntervals they were remo ed and the pl cental tissue was washed 5 times with 10—15 ml cold physiological saline

In vivo experiment

In one e periment iron transf from matern l plasma to the fetuses was measured on the twent th day or pregnancy in 5 control rats and in 5 rats in wh ch the plasma transferrin concentrat on had been in reased to approx mately twice the ontrol alue by the injection of transferrin Th ats were anesthet ed l ghtly with ther and we e injected by th saphenous vein with 1 0 ml physiolog cal sal ne (controls o with transferrin dissolved in 1 0 ml sal ne Th iron had ea l er been removed f om tl transferr n b p l filtration Fifteen minutes after the sal e or tr nsf rrin nject o is 5 µ Fe (10 µc pe / as ferrous sulphate was injected slowl by th same e n Ex ctly 1 n lat the r t s abdomen w pened and th f tuses were emoved und eth anesthesia A sampl of m tern l blood was obtained by heart pun ture t the same t me Th fetuses wer th n washed w th sal ne homogenized n a W ng bl nder and m d up to m a ured lum with sal n Th ee nl samples of the homogen tes samples f the m t rn l pla na nd st d rds ma t f n th njected olution w counted f r r d o ct t I n ra sf r t tl f tuses d mat rnal plas a r n turnov w r cal ulated from thes v lues f th m u ed n ternal s rum r n n entrat n nd from a plasm olume of 5 0 ml pe 100 g bod w gl t B n l 1918) It w umed that tl ro njected as F d d not gn f il lt the plasm o entrat n

Analyt al m thod Seru n n l tot l o b n l g cap ty (TIBC wh h was used to mea ure tr n f t on were d t rm ned b a m ro- daptat on f tl e m tl od of Morgan nd Ca t 1960 R d ct ty w m asured n a w llype sc t llat n d tector Th two isotopes I I when p esent in san e sam pl were d ff rent ated by a pulse he ght analys s.

TABLE I Transferrin and iron uptake by rat allantoic and yolk sac placenta when incubated for 30 minutes in solutions of constant iron concentration but varying transferrin concentration (TIBC) Purified transferrin was used in experiment 1 and fresh whole plasma in experiment 2

Exp	Iron concentration (μg/100 ml)	TIBC (μg/100 ml)	Transferrin bound (μmoles/g × 10)		Iron uptake (μmoles/g × 10)	
			Allantoic	Yolk sac	Allantoic	Yolk sac
1	30	760	3 28	6 06	0 55	1 30
	30	250	1 24	2 36	0 52	1 37
	30	40	0 25	0 32	0 56	1 33
2	140	450	—	—	2 07	7 71
	140	300	—	—	2 06	7 79
	140	150	—	—	2 03	7 83

TABLE II Effect of enzyme inhibitors (concentration 5×10^{-3} M) on iron uptake by allantoic and yolk sac placenta in the rat The results are expressed as the mean of 10 observations ± standard error of the mean

Inhibitor	Iron uptake (per cent of control value)	
	Allantoic	Yolk sac
Sodium cyanide	81 ± 5 8	58 ± 12 6
Sodium fluoride	94 ± 6 7	69 ± 8 7
Sodium arsenite	94 ± 10 6	38 ± 13 1
2 4-dinitrophenol	109 ± 8 7	59 ± 9 8

Results

Transferrin and iron uptake by rat placenta in vitro Comparison of allantoic and yolk sac placenta When placental tissue was incubated with transferrin ^{131}I and ^{59}Fe a progressive uptake of both isotopes occured However whereas placental uptake of transferrin was initially very rapid and then slowed down to almost a plateau level by 30 minutes iron uptake continued in a linear fashion throughout the incubation period (Fig 1) The amount of transferrin bound per g placental tissue was approximately twice as great and the rate of iron uptake was 2—4 times as great with yolk sac placenta as with allantoic placenta (Fig 1 Table I)

Effect of iron concentration When iron concentration was varied but transferrin concentration was maintained constant transferrin uptake by both types of placenta remained constant while iron uptake increased with increasing iron concentrations between 30 and 320 μg per 100 ml The results of one experiment are shown in Fig 1 In

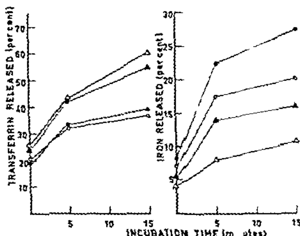


Fig 2 Release of transferrin and i on f om rat allantoic (● ○) and yolk sac placenta (▲ △) in vit when reincubated in rat plasma (● ▲) or sodium chlor de bicarbonate solution (○ △)

this experiment transferrin uptake was the same whether the protein was only 6 per cent or was 100 per cent saturated with iron.

Effect of transferrin concent ation Incubation of placental tissue in solutions of constant iron concentration but increasing transferrin concentrations resulted in increased uptake of transferrin but no change in iron uptake (Table I)

Effect of temperature When incubation was performed for 30 min at 3 C transferrin uptake by allantoic and yolk sac placenta respectively was 35 and 25 per cent of the values obtained on incubation at 37 C Similarly iron uptake was depressed to 30 and 15 per cent respectively of the 37 C values

Effect of en yme inhibitors Iron uptake by yolk sac placenta was depressed to 40—70 per cent of control values by sodium cyanide sodium fluoride sodium arsenite or 2 4-dinitrophenol (concentration 5 10 M) However only sodium cyanide was found to have a significant effect on iron uptake by allantoic placenta

Incorpo ation of i on into ferritin When placental tissue was incubated for 30 minutes at 37 C with transferrin bound Fe the mean values of 10 determinations for the percentage of placental radioactivity which was precipitated by anti horse ferritin serum were 2 8 (standard error of the mean 0 35) and 1 35 (± 0 38 for allantoic and yolk sac placenta respectively

Release of transferrin and on f m ra p'acen a in t t When placental tissue which had taken up transferrin ^{131}I — Fe was reincubated in either plasma or sod um chloride bicarbonate solution at 3 C both isotopes were prom ex vely released Fig 2) Transferrin release was more rapid and on r l ase less rapid with yolk sac than with allantoic placenta. With both t pes f placenta relatively more transferrin than iron was released during th 15 m n t e ncubation time The rate of release of t ansferrin was almost the sam wh n th r cubation was performed in plasma or in sodium chlor de bi carbonate solution However iron reflux was def nitely greater into plasma than into the salt sol tit n

In other reflux experiments the following compounds were added to the reincubation plasma inos l oncentration 8 10 M) hypoxanthine (4 10 M) manganous

TABLE III Iron transfer in 15 minutes from maternal plasma to fetuses on the twentieth day of pregnancy in rats injected intravenously with physiological saline (control rats) or with rat transferrin

Rat no	Maternal body weight (g)	No of fetuses	Plasma iron concentration (μg/100ml)	TIBC (μg/100ml)	Maternal plasma iron turnover (μg/min)	Iron transfer to fetuses (μg)
Control rats						
1	230	10	105	355	0 58	1 25
4	255	7	102	450	0 65	1 61
5	275	10	87	371	0 68	2 86
7	260	7	131	351	0 72	1 21
10	235	8	193	357	0 96	2 23
Mean	251	8 4	136	377	0 80	1 83
Transferrin injected rats						
2	240	10	128	729	0 52	1 2
3	240	7	107	954	0 45	1 4
6	250	8	274	681	1 09	1 4
8	250	10	136	712	0 86	2 5
9	235	7	165	983	0 77	2 3
Mean	243	8 4	162	812	0 74	1 8

chloride (8 × 10 M) 2 amino-4 hydroxypteridine 6 aldehyde (2 μg per ml) 2 4 dinitrophenol (5 × 10 M) and sodium arsenite (5 × 10 M) None of these compounds affected the amount of iron released during 15 and 30 min reincubation

Placental transfer of iron in vivo Effect of plasma transferrin concentration Table III shows the results of the experiments in which iron transfer from maternal plasma to the foetuses was measured in control rats and in rats with increased plasma transferrin levels It can be seen that doubling of the plasma transferrin concentration did not affect either the amount of iron transferred to the foetuses or the calculated rate of plasma iron turnover

Discussion

The passage of transferrin or β globulins from maternal to fetal plasma (Dancis and Shafran 1958 Bangham 1960 Kulangera and Schjeide 1962 Morgan 1964 a) is much smaller than that of iron Hence the iron must be removed from maternal plasma transferrin during iron transfer to the foetus Bothwell *et al* (1958) showed that if fetuses were removed but placentae were left in position in the uterus of the rabbit the placentae continued to take up iron from maternal plasma at the same rate as iron was transferred to the foetuses in control rabbits with the fetuses in situ *In vivo* then the placenta itself

has the ability to take up iron from transferrin The present experiments have shown that the sliced rat placenta *in vitro* retains this ability Furthermore on a weight basis the yolk sac placenta takes up iron more rapidly than does the allantoic placenta Although transferrin itself is bound to the placenta in this process the uptake of iron during 30 min incubation with both types of placenta was 2—3 times as great as could be accounted for simply by binding of the transferrin iron complex

The results of the experiments where iron concentration was varied with constant transferrin concentration indicate that the rat placenta *in vitro* binds transferrin molecules equally well whether or not they contain iron Varying the transferrin concentration with constant iron concentration showed that iron uptake by placenta is independent of the transferrin concentration or the degree of saturation of transferrin with iron The increased iron uptake found when the iron concentration of the incubating solution was increased must therefore have been the result of the change in iron concentration and not of that in per cent saturation of transferrin with iron In several respects iron uptake by the rat placenta *in vitro* resembles that by rabbit reticulocytes which have been found to take up iron independently of the transferrin concentration and to bind transferrin equally readily whether or not it is complexed with iron (Morgan and Laurell 1963 Schade 1964) It has been shown that iron uptake by rat reticulocytes is inhibited by low temperature and by enzyme inhibitors (Jandl *et al* 1959 Morgan 1964) suggesting that the processes required energy derived from cell metabolism The present results indicate that the same is probably the case with the placenta Although all the enzyme inhibitors used caused diminished iron uptake by the yolk sac placenta only sodium cyanide significantly affected that by allantoic placenta This difference may be related to the much greater rate of iron uptake by yolk sac than allantoic placenta

In the intact animal the placenta passes on the iron aquired from maternal plasma to the fetus In addition if the transferrin bound to the placenta is neither catabolised or transmitted to the fetus it must be released and returned to maternal blood plasma The present experiments show that release of transferrin and iron do also occur *in vitro* Transferrin release occured almost as readily when placental tissue was reincubated in sodium chloride bicarbonate solution or in plasma Iron release was however less when transferrin was absent from the reincubation medium It therefore appears that the release of transferrin from the placenta does not require the presence of transferrin molecules in the surrounding medium but that iron release may do so

It has been shown (Wöhler 1955) that radioactive iron injected intravenously into the pregnant rabbit is incorporated into ferritin in the placenta This suggested that the iron passing from maternal plasma to the fetus may be incorportaed into ferritin during its passage through the placenta Ferritin has been crystallized from the rat placenta (Nylander 1953) However in the present work the fraction of placental radioactive iron which could be precipitated with antiferritin serum was extremely small This suggests that if iron uptake *in vitro* occurs by the same mechanism as in the living animal little of the iron passing through the placenta is incorporated into ferritin If placental iron transfer did involve incorporation into ferritin then it is conceivable that the mechanism of its release from ferritin before being transferred to fetal plasma involved reduction of the iron by xanthine oxidase (Green and Mazur 1957 Mazur Green and Saha 1958) It was found however that neither xanthine oxidase substrates (hypoxanthine inosine) or inhibitors (2 amino-4 hydro-xypteridine-6-aldehyde and manganous chloride) had any effect on iron release from the placenta *in vitro*

The results of the *in vivo* experiments showed that doubling of the plasma transferrin concentration was not accompanied by *any change in iron transmission to the fetuses*. This confirms the *in vitro* finding that iron uptake by the placenta was independent of the transferrin concentration. Hence there is no evidence of competition between the placenta and maternal plasma transferrin for its iron. Therefore any change in plasma transferrin concentration during pregnancy should *not impair iron* uptake by the placenta and iron transfer to the fetuses in the rat. The transferrin concentration decreases during pregnancy in the rat (Morgan 1961 b) but by contrast it increases markedly in humans (Laurell 1947) and rabbits (Morgan 1961 b). If the mechanism of iron exchange between transferrin and placenta is similar in humans and rabbits to that in rats the elevated transferrin level will not affect placental transfer of iron. Furthermore the results of experiments on iron uptake by reticulocytes (Morgan and Laurell 1963, Schade 1964) indicate that it will not be expected to impair iron uptake by erythropoietic tissue. It is possible that the high transferrin concentration of late pregnancy in these two species may aid iron transfer to the placenta and erythropoietic tissue by impairing iron deposition in and facilitating its mobilization from the iron stores.

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Acta physiol scand 1964 62 280—286

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Reflex Influence of "Somatic Pressor and Depressor Afferents" on Resistance and Capacitance Vessels and on Transcapillary Fluid Exchange

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Abstract

Johansson B O Lundgren and S Mellander *Reflex influence of somatic pressor and depressor afferents on resistance and capacitance vessels and on transcapillary fluid exchange* Acta physiol scand 1964 62 280—286 — The reflex circulatory effects of afferent somatic nerve stimulation *were studied in the different consecutive vascular sections in skeletal muscle and intestine* In skeletal muscle the somatic pressor reflex elicited constriction of resistance and capacitance vessels and increased the pre /postcapillary resistance ratio while precapillary sphincter tone was unchanged In the intestine there was reflex constriction of capacitance and precapillary sphincter vessels but only transient changes in the resistance function In a situation of increased *vasoconstrictor fiber activity produced by carotid occlusion stimulation of somatic depressor afferents elicited* dilatation of resistance and capacitance vessels in both skeletal muscle and intestine Carotid occlusion produced continuous absorption of fluid from the extravascular space in skeletal muscle due to a fall in capillary pressure A superimposed stimulation of somatic depressor afferents was able to restore capillary pressure to the control level despite incomplete restoration of total resistance and capacitance functions A dilatation of precapillary sphincters occurred in intestine but apparently not in skeletal muscle during the somatic depressor reflex

Peripheral somatic nerves are known to contain afferent fibers which make reflex connections *with vasomotor neurones within the central nervous system* An excitatory influence is exerted by the non medullated C fibers while inhibitory effects are elicited mainly *by the group III muscle* afferents (Laporte and Montastruc 1957 Laporte Bessou and Bouisset 1960 Skoglund 1960 Johansson 1962) The circulatory response patterns associated with the pressor and depressor reactions produced by stimulation of these afferents have been analyzed previously in terms of blood flow changes in different parallel coupled vascular circuits such as skeletal muscle skin intestine and kidney (Johansson 1962) It was shown that stimulation of somatic pressor

afferents produced a vascular response pattern which was differentiated with regard to the various organs and which differed from the reflex adjustments induced by carotid occlusion The "somatic depressor reflex was found to be associated with an inhibition of the sympathetic vasoconstrictor fiber tone in all vascular regions studied and a reflex inhibition of heart activity

To elucidate in more detail the functional significance of these reflexes the analysis of their response patterns should include studies not only of the resistance function but also of the capacitance function and of the capillary fluid transfer in the vascular beds Such information would be of interest since redistributions of intra and extra vascular fluid volumes are of fundamental importance in cardiovascular homeostasis Some preliminary experiments (Johansson 1962) suggested that a reflex dilatation of intestinal capacitance vessels was part of the circulatory response pattern in the somatic depressor reflex The aim of the present investigation was to study in more detail the reflex influence of the somatic pressor and depressor afferents on the pre and post capillary resistance vessels on the precapillary sphincters and on the capacitance vessels in skeletal muscle and intestine

Methods

Experiments w e perform d on 8 cats l ghtly anesthetized w th chlo alose (30 to 40 mg kg) The reactions of the consecut e v scular sections were stud ed in a sk letal muscle preparation of the h nd part of the cat and n a p eparation of small intestine The details of the experimental technique and the method of analys ng the recordings are described elsewh re (Mellander 1960 Folkow Lundgren and Wallent n 1963) Blood flow was measu ed by a drop recorder unit connected to the v n draining the region stud ed Arte al inflow pressure was monitored from the inferior mesenteri artery By these means the reactions of the resistance vessels could be followed continuously The arterial perfusion pressure could be kept constant during cir ulatory reflex engagements by adjust ng a sc ew clamp placed on the abdom nal aorta The tissue with ntact blood supply and vasomoto fibe innervat on was enclosed n a pl thysmograph to perm t ecord ng of hanges n tissue volum Alterations n the volume of blood contained n the capa itance vessels produ d changes in tissue volume which could be distinguished f om those caused by net m ement of fluid across the cap llary walls (see Mell nder 1960) The ma n pa t of the respons n the capacitance vessels was hown to be du to act e changes in their smooth muscle tone while a minor part was due to changes in transmural pressu e with n th s section By determination of the capillary filtrat on coefficient (CFC) changes n the s ze of the cap llary surface area ava lable for exchange could be deduced and by this also changes in the tone of the p ecap llary sph ncters (see Cobbold *et al* 1963)

Somatic afferent nerve fibers in the brachial pl xus we e stimulated B pol r s l er electrodes and a Crass t mulato (mod 1 4 E) wer used The somat depressor reflex was el ted by st mul of 2—5 V mpulse duration 1—1 5 msec and f equency 5—10 mp sec Pressor responses w e evoked w th 10—15 V ?—3 msec and 20—30 imp sec

The an mals we e at first given artifi ial ent lat on to a level wh ch just suppressed spontaneous esp rat on and then to a o d influ n of reflex changes in skeletal muscl tone upon th rculatory system giv n gallam n Fla ed l 1—4 mg kg i v

Card ov scular adjustements associated with ch nges n arterial blood p essure are to a great extent m d fied in the effecto espon p tterns b th secon lary buffering nflu n e of the baro eceptor reflexes Such nfluence has been h wn to interfer ma kedl w th the rea t on of th es tance ess ls el cited f om the s mat depresso and presso afferents (Joh nsson 19? Tl primary response pattern of the latter eflexes we e more clearl revealed if th buff ng act on of the baro eceptors was redu ed o el m nated This wa also accompl l ed n m ost of the present exper ments by secti n ng the agi at the cerv cal lev l and lamping the o mon carot d rteries Furth r these procedures raised th level of t n c tivity in th sympat t asoconst ctor f bers so tl t th ympatl o nh b tory act on of th somat c dep essor afferents could be mor clearly revealed

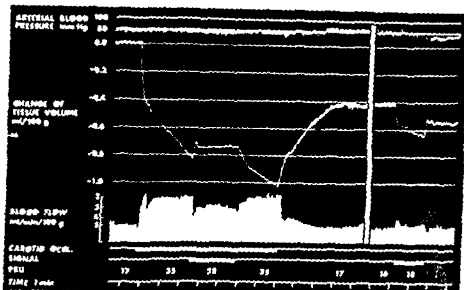


Fig 1 Vascular effects in cat skeletal muscle of left panel carotid occlusion and stimulation of *somatic depressor afferents and of right panel stimulation of somatic pressor afferents Carotid occlusion elicits constriction of resistance vessels (reduction in blood flow) and of capacitance vessels (initial steep decline of tissue volume) and an absorption of extravascular fluid to the circulation (slow continuous decline in volume Superimposed depressor afferent* stimulation (signal) produces a moderate dilatation of resistance and capacitance vessels (*increase of blood flow and rapid increase of volume*) and a complete cessation of the prevailing fluid absorption (tissue volume stays constant after initial *rapid increase*) – Stimulation of somatic pressor afferents elicits constriction of resistance and capacitance vessels and absorption of extravascular fluid

Results

The first section of Fig 1 illustrates the effects on tissue volume and blood flow in skeletal muscle produced by occlusion of the common carotid arteries and by stimulation of somatic depressor afferents while the arterial inflow pressure was kept constant It can be seen that carotid occlusion elicits a pronounced and well sustained effect in the resistance vessels as judged from the change in regional blood flow Regional resistance to flow (PRU) increases from 17 to 35 The tissue volume is constant (isovolumetric) in the control period On carotid occlusion there is an initial rapid reduction of volume followed by a somewhat slower but continuous decline As has been established previously (e g Mellander 1960) the first phase coordinated in time with the phasic response of the resistance vessels represents the amount of blood expelled from the capacitance vessels The second phase reflects an absorption of fluid from the extravascular space into the circulatory system and this fluid becomes part of the venous outflow The absorption phenomenon is due to a fall in mean capillary hydrostatic pressure secondary to a reflex increase of the pre- to postcapillary resistance ratio (Mellander 1960) In the experiment of Fig 1 carotid occlusion produced an expulsion of about 0 4 ml blood per 100 g tissue from the capacitance vessels and an absorption of extravascular fluid at a rate of 0 16 ml/min 100 g CFC was determined

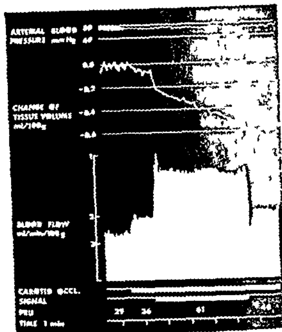


Fig 9 Vascular effects in skeletal muscle after hemorrhage of carotid occlusion and of stimulation of somatic pressor afferents (signal) The pressor reflex is associated with constriction of resistance and capacitance vessels and with absorption of extravascular fluid (cf Fig 1) Here the buffering capacity of the baroreceptors is reduced by the carotid occlusion and preceding hemorrhage and the effects of the somatic pressor reflex are greater especially on the resistance vessels

in this experiment during another period of carotid occlusion There was an initial slight and transient decrease in CFC but within a few minutes it rose to values slightly above control This indicates a temporary reflex constriction of the precapillary sphincters followed later by a dilatation (cf Cobbold *et al* 1963) During the steady state period of carotid occlusion CFC amounted to 0 019 ml min 100 g mm Hg From this value of CFC and from the observed rate of fluid absorption it can be calculated that the reflex fall in mean hydrostatic capillary pressure amounted to about 8 mm Hg during the carotid occlusion

Somatic depressor afferents were stimulated (signal) at the stage when the vascular adjustements to carotid occlusion had reached a steady state The stimulation elicited a reflex fall in regional flow resistance from 35 to 78 PRU and simultaneously a slight increase in regional blood volume After this capacitance response the volume curve became isovolumetric for the remainder of the stimulation period This indicates that there was no net movement of fluid across the capillary walls Since there seemed to be no significant change in CFC in the somatic depressor reflex the findings suggest a return of mean hydrostatic capillary pressure to the level prevailing in the control period i e a rise of about 8 mm Hg The return to the isovolumetric state which was almost invariably observed during somatic depressor afferent stimulation deserves special emphasis in view of the fact that the resistance and the capacitance functions were not restored to control levels

After cessation of the somatic depressor afferent stimulation the effects of the carotid occlusion on the resistance and the capacitance vessels were regained There w...

reappearance of transcapillary fluid absorption although at a slower rate Release of the carotid occlusion resulted in a virtually complete recovery of the resistance and capacitance functions After this the volume curve is seen to approach the isovolumetric state This indicates that there was as expected no definite rise in hydrostatic capillary pressure above the control level which explains why the loss of extravascular fluid is not immediately replaced

The right panel of Fig 1 illustrates the effects of stimulation of somatic pressor afferents The carotid arteries were not clamped prior to this timulation Regional arterial pressure was kept constant The vascular response pattern in skeletal muscle is similar to that seen during carotid occlusion in so far as there is a constriction of resistance and capacitance vessels and an absorption of extravascular fluid but the effects are less pronounced This may in part be due to a concomitant buffering in fluence of the intact baroreceptors caused by the reflex increase in arterial blood pressure of about 50 mm Hg occurring in the main circulation during pressor afferent stimulation

This buffering function was reduced in the experiment shown in Fig 2 by bleeding the animal cutting the vagi in the neck and clamping both carotid arteries The bleeding produced a reflex constriction of the resistance and capacitance vessels and a transcapillary absorption of fluid The continuous decline in volume thus obtained was compensated for by raising venous outflow pressure and thereby capillary pressure until the volume curve became isovolumetric This is the situation in the control period of Fig 2 Under these circumstances carotid occlusion elicited vascular responses that were much less pronounced than those obtained in Fig 1 Evidently the carotid reflex mechanisms were already engaged to a great extent in the response to bleeding Stimulation of the somatic pressor afferents was now able to produce a clear cut constriction of the resistance and the capacitance vessels and an increase of the pre to postcapillary resistance ratio as judged from the continuous absorption of extra vascular fluid All these effects were well maintained during the period of stimulation There was an especially pronounced constriction of the resistance vessels while the magnitude of the capacitance response was approximately the same as in the somatic pressor response in Fig 1 An explanation for this may be that in the experiment of Fig 2 the relatively high level of sympathetic activity prevailing before the stimulation had produced a vasoconstrictor response which was closer to maximum in the capac itance than in the resistance vessels This is dependent upon the different shapes of the frequency response curves for these two vascular sections (see Mellander 1960) Stimulation of the somatic pressor afferents did not seem to alter CFC in skeletal muscle significantly suggesting that a sustained constriction in the precapillary sphinc ter section was not obtained

In the intestinal vascular bed stimulation of somatic pressor and depressor afferents produced reflex effects on the resistance and capacitance vessels imilar to those found in skeletal muscle although quantitatively less pronounced Sustained reflex changes in the pre to postcapillary resistance ratio regularly observed in the skeletal muscle did not occur in the intestine This was judged by the fact that the reflex adjustments were not associated with net movement of fluid across the intestinal capillary walls Further in contrast to skeletal muscle there was in the intestine a small but sustained increase in CFC upon stimulation of somatic depressor afferents and conversely a sustained decrease during the somatic pressor reflex

Discussion

The somatic pressor and depressor reflexes have been looked upon as somatomotor reactions to different kinds of nociceptive stimuli (Johansson 1962) The pressor reflex was considered as an autonomic component of the excitatory behavioural response to cutaneous pain while the depressor reflex was believed to be associated with the immobilizing and nauseating type of deep pain It is possible therefore that these reflexes become functionally significant only under certain pathophysiological conditions Further as illustrated also in the present experiments their circulatory effects will become pronounced only in situations when the functional capacity of the cardiovascular buffer mechanisms is reduced

The somatic pressor reflex is characterized by quantitative differences in the reactions of the different parallel-coupled vascular circuits so that for example the resistance response in the kidney is more pronounced than in the skeletal muscle (Johansson 1962) The pattern of response of this reflex with regard to the various consecutive vascular sections was studied in the present experiments In skeletal muscle there was a clear-cut constriction in the resistance and capacitance vessels an increased pre to postcapillary resistance ratio but no significant effect on the precapillary sphincters In the intestine there was a constriction of the precapillary sphincters and of the capacitance vessels but only transient constrictor effects were obtained in the resistance vessels and the pre to postcapillary resistance ratio did not change These patterns of vascular response in skeletal muscle and intestine are very similar to those obtained on direct regional vasoconstrictor fiber stimulation (Mellander 1960 Folkow *et al* 1964 a b) The different reactions in the consecutive vascular sections are consequently not specific for the somatic pressor reflex and do not seem to be due to a differentiation of the vasoconstrictor fiber discharge to the series-coupled sections but appears to be related to local vascular adjustments (Cobbold *et al* 1963 Folkow *et al* 1964 a)

As mentioned above the effects obtained on somatic pressor reflex engagement are pronounced and sustained only when the counteracting influence of the baroreceptors is minimized Such conditions may prevail after trauma associated with blood loss and pain The nociceptive stimulus may then reinforce reflexly the activity in the sympathetic vasoconstrictor fibers thereby augmenting the peripheral resistance and increasing venous return The later effect would be due partly to constriction of capacitance vessels and partly to absorption of extravascular fluid

The somatic depressor reflex is associated with a generalized inhibition of sympathetic vasoconstrictor fiber activity to the resistance vessels in various tissues (Johansson 1962) The present study has shown that this reflex adjustment includes also a dilatation of the capacitance vessels in skeletal muscle and intestine The net fluid absorption which was reflexly induced in skeletal muscle by carotid occlusion was completely cancelled by stimulation of the somatic depressor afferents This must mean that the ratio of pre to postcapillary resistance which was increased by the carotid occlusion was returned to control level by the stimulation This precise adjustment of the pre and postcapillary resistances with restoration of mean capillary hydrostatic pressure may suggest a rather specific differentiation with regard to the effects on the various consecutive vascular sections in skeletal muscle since simultaneously the total resistance function and the capacitance function were not returned to control values The possible existence of such a specific reflex mechanism for the control of transcapillary fluid exchange is an interesting but complex problem which deserves further investigation.

It is apparent that the reflex effects elicited by the somatic depressor afferents can profoundly affect the general cardiovascular dynamics It has been pointed out previously that the inhibitory action of this reflex on the resistance vessels and on the heart could under certain circumstances result in a circulatory collapse similar to that seen in some conditions of deep pain (Johansson 1962) The changes in the circulatory blood volume produced by reflex dilatation of capacitance vessels and by abolition of prevailing extravascular fluid absorption add to this general picture of circulatory derangement Whether this reflex is of pathophysiological significance only or if it is engaged in normal cardiovascular control remains to be revealed

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The Influence of Chemically Pure Secretin on Hepatic Bile Output

By

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Abstract

Jonson G L Sundman and L Thulin *The influence of chemically pure secretin on hepatic bile output* Acta physiol scand 1964 62 287—290 — In awake cholecystectomized dogs with a permanent external common duct fistula the bile output increased during infusion of a secretin preparation containing about 20 000 clin units per mg of substance (Jorpes and Mutt 1961) At the largest dose about 43 clin units per kg b wt and hour it was about 3 times the initial output and even then it was smaller than the output during digestion of food with bile The relation between the log of the secretin dose and the bile output was linear

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Preparations containing from a few up to 4 000 clinical units of secretin per mg of substance have been used in earlier studies of the choleretic effect of secretin (Bayliss and Starling 1902 Ågren 1934 Ågren and Lagerlof 1937 Grossman *et al* 1949 Werner and Mutt 1954 Thomas 1950) Later a chemically pure preparation containing about 20 000 clinical units per mg of substance has been made (Jorpes and Mutt 1961 Jorpes Magnusson and Steele 1962) We have investigated the effect of this preparation on the hepatic bile output

Material

Experiments were performed on two male mongrel dogs They were cholecystectomized and provided with a permanent external common bile duct fistula of a type with minimal risk of biliary stasis and infection (two-part fistula Jonson 1964 a b) The relative standard deviation of 24 hour bile output is about 5 per cent in such dogs with two-part fistulas at standard conditions The dogs displayed no evidence in plasma of biliary stasis during the experimental period They ate a constant amount of standard food at 12 o clock every day the food was mixed with all the bile produced during the previous 24 hours

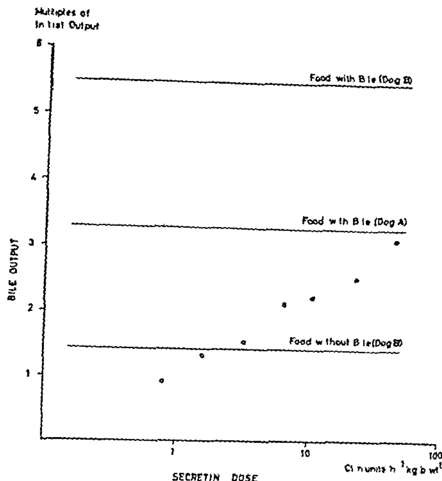


Fig 1 Hepatic bile output related to secretin dose Initial output is the output immediately before secretin infusion in each experiment ○ dog A ● dog B

Procedure

All 11 experiments were begun at meal time The dogs were stood in a Pavlov frame where they always took their food immediately when given

The secretin was administered through a vein catheter introduced at least half an hour before the experiment It was dissolved in sterile saline immediately before the infusion and 90 ml of the solution were given per hour A new solution was made at least every hour The secretin dose ranged from 0 67 to 43 clinical units per kg b wt and hour The infusion was intermittent (1 expt in dog A) or continuous with a stepwise doubled dose of secretin (1 expt in dog A 4 in dog B)

Each experiment started with 2 hours of fasting during which the bile output was measured It was then in 5 control experiments measured during 8 hours of either (1) prolonged fasting (1 expt in each dog) (2) digestion of food with bile (1 expt in each dog) or (3) digestion of food without bile (1 expt in dog B) In 6 experiments the output was measured during 6 hours of secretin infusion and during two hours after the infusion The end of the fistula was placed at the level of the xiphoid process of the dog The bile was collected from the fistula into a volumetric vessel graded in tenths of a ml and was measured every half hour

TABLE I Bile output during two-hour periods in 2 experiments of secretin infusion on dog A

Infusion method	Bile output: Before secretin (ml)	Clin. secretin units/hr/kg b. wt: 0.78	1.56	3.12	6.24	After secretin
Intermittent	4.6	0.9	—	1.4	—	*0.7
Continuous	3.0	—	1.3	1.8	[illegible]	0.7

Mean of 3 last half hours in each period

Output during and after secretin infusion is expressed in multiples of output before infusion.

TABLE II Bile output during two-hour periods in 4 experiments of continuous secretin infusion on dog B

Before secretin (ml)	Clin. secretin units/hr/kg BW: 0.67	1.34	2.68	5.36	10.72	21.44	42.88	After secretin
1.5	1.3	1.1	[illegible]	—	—	—	—	0.3
1.0	[illegible]	0.9	1.3		—	—	—	0.8
[illegible]	—	—	1.3	1.8	[illegible]	—	—	0.6
1.7	—	—	—		2.3	[illegible]	3.1	0.1

Output during and after secretin infusion is expressed in multiples of output before infusion.

All experiments were divided into two-hour periods and in each period the bile of the first half hour was omitted. Thus bile output values during transitional stages were consistently omitted. The average output during the initial two-hour period was expressed in ml per half hour. The average output during each succeeding two-hour period was expressed as multiples of the initial output in the same experiment.

Results

Fasting

The initial output was 3.0 and 1.3 ml per half hour in dogs A and B respectively. During 4 two-hour periods the output gradually decreased to 0.9 and 0.6 of the initial one.

Digestion

The initial output was 3.3 and 1.5 ml in dogs A and B respectively. During digestion of *food with bile* the output increased to 3.1—3.6 (mean 3.3) and 5.0—[illegible] (mean 5.5) times the initial one. During digestion of *food without bile* the output increased from the initial output of [illegible] ml to 1.3—1.5 times this value (mean 1.4, dog B).

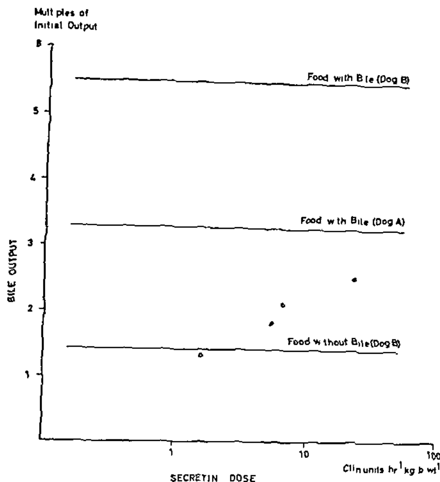


Fig 1 Hepatic bile output related to secretin dose Initial output is the output immediately before secretin infusion in each experiment ○ *dog A* ● *dog B*

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Acta physiol scand 1964 62 291—292

From the Department of Histology and Clinic of Ophthalmology University o [illegible] eden

Distribution of Adrenergic Nerves to Orbital Structures

By

BERNDT EHI GER

Knowledge of the distribution of the adrenergic nerves in the eye and related orbital structures is important for understanding the physiology of the eye not least with respect to the possibility that adrenergic mechanisms might play a role in the regulation of the homeostasis of the eye (cf Bárány 1962 Sears & Sherk 1964) The advent of a highly sensitive fluorescence method for the histochemical demonstration of adrenergic nerves (Falck 1962) has stimulated studies on the distribution of such nerves in the eye and related orbital structures

Eye segments from albino mice and rats and from randomly pigmented guinea pigs rabbits and cats were treated for fluorescence microscopy according to Falck (1962) The retina was not included in this study since it is the object of a current investigation by Malmfors (cf Malmfors 1963)

The cornea has a sparse network of adrenergic nerves The amount varies however between different animals even within the same species The corneal epithelium appear to be devoid of adrenergic nerves In the *anterior chamber angle* of guinea pig the trabeculae have a rich supply of adrenergic varicose fibres whereas in mouse rat rabbit and cat there are few such nerves in this region. However at irregular intervals there appear in these latter species dense accumulations of fluorescent nerves which are seemingly associated with the vessels The *ciliary body* has an abundant supply of adrenergic nerves mainly situated subepithelially The ciliary muscle of guinea pig contains a network of very fine varicose fibres in the other species this is not so conspicuous In rabb t nerve fibre bundles can be found running equatorially with branches issuing radially into the iris In the *ciliary processes* adrenergic nerves follow the vessels in the form of a plexus In the *iris* the majority of the adrenergic nerves are concentrated to the dilatator muscle from where branches issue anteriorly to form a wide meshed network. The sphincter pupillae contains remarkably many fluorescent fibres in cat.

In the *choroid* the fluorescent nerves are mainly associated with the vessels but a fine lattice chiefly of varicose fibres runs freely in the stroma The *sclera* contains no adrenergic fibres except where vessels penetrate enclosed by the ordinary vascular plexus

In the *nictitating membrane* of cat the muscular part contains a dense network of adrenergic nerve fibres The membraneous parts contain very few fluorescent fibres

Infusion of secretin (Fig 1)

In dog A the initial output in 2 expts was 4 6 and 3 0 ml It did not increase when 0 78 clin units of secretin per kg b wt and hour were infused but doubled when 6.24 units were given (Table I) After the end of the infusion the output decreased to 0 7 of the initial output

In dog B the intial bile output in 4 expts was between 1 0 and 1 7 ml (mean 1 5 ml) When the secretin dose was stepwise doubled from 0 67 to 43 clin units per kg b wt and hour the bile output increased from 1 3 to 3 1 times the initial output (Table II) After the end of the infusion the output decreased to 0 1—0 8 of the initial one

Discussion

The results indicate that a secretin dose presumably giving a pancreatic response as large as that of an ordinary meal gives only a moderate increase of bile output The output seems to be equal to that after a meal without bile Thus during normal digestion the choleretic action of secretin seems to be small compared with that of bile acids

When the secretin dose was increased as here from one to about forty clinical units per kg b wt and hour the bile output increased asymptomatically to three times the initial output There was a semilogarithmic relation between the secretin dose and the bile output the output expressed as a multiple of the initial output was directly related to the logarithm for the dose (Fig 1) Such a clear relation between them has not earlier been established

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From the Institute of Physiology University of Uppsala Uppsala Swed

The Ion Flux Across Membranes During Electro-Diffusion and Convection

By

TORSTEN TEORELL

When a membrane containing *wide* pores separates two compartments (1) and 2) each containing a single salt (such as NaCl) at different concentrations C_1 and C the fluxes of the cation and anion are dependent on these concentrations on the ion mobilities u and v and on the driving forces due to a) the concentration gradient b) the transmembrane potential (E) gradient and c) the linear bulk flow velocity (V) through the membrane pores (convection) The first flux formulation comprising the forces a) and b) i e "electro-diffusion flux was given already in 1897 by Behn Below a formula for the cation flux will be derived for electro-diffusion and con vection i e the case where all the three forces a) b) and c) are present The derivation will be adapted to conform with the conditions of the membrane oscillator' previously described (Teorell 1959 a b) and directly given a form that can be employed in experiments or in analog computation

In the *differential* form the flux equation for the cation can be written (see, for instance Teorell 1951 Eq 15 and Teorell 1953 Eq 5 1)

$$\text{Flux} = -Uc \left[\frac{RT\ d(\ln C\ )}{d} + F\ \frac{dE}{dx}\right] - C_x V \tag{1}$$

A steady state is assumed hence the equation is valid at any cross section d of the membrane with the thickness δ ($0 < x < \delta$) Obviously the term with the bracket is the electro-diffusion and the $C\ V$ term the convection contribution to the flux Rearranging (1) to introduce the cation transference number $/F^2 (u + v)$ leads to

$$Fl\ \ = -\frac{u}{F^2(\ \ + v)}\ \frac{[RT\ d(\ln C_x\ + FdE\ - (V\ d\ \ ]}{\dfrac{dx}{F\ (\ + v)\ C_x}} \tag{2}$$

Now integration of the numerator and denominator can be performed between $= 0$ and $x = \delta$ The $\int^{\delta} dx/F^2 (u - \ ) C$ is in fact the *total* (integral) *transmembrane resistance* denoted R^{∞}_{tot} because the $F^2 (u + v)\ C_x$ is the *conductance* of the membrane 1 ce *d* The *integrated* flux formula becomes finally

$$Fl\ x = \frac{u}{F\ (\ +\ )}\ \frac{[RT\ \ln C\ C\ ) - F\ E - F\ \ - V\delta u]}{[R^{\infty}_{to}\ ]} \tag{3}$$

The terms $RT\ \ln(C\ C\ )$ and $F(E\ E\ )$ are respectively the chemical and electric *work* terms. The convection term $V\delta$ has also the dimension of work because the cation mobility

u is actually defined as $u = u/F$ (where u is expressed in cm/sec/volt and F is the Faraday constant) Hence Eq (3) can be transcribed in words as

$$\text{Cation ion flux} = \frac{1}{F} \frac{(\text{cation transference number})\ (\text{sum of work terms})}{(\text{total membrane resistance})} \tag{4}$$

An explicit solution of $R^{\infty}_{tot} = f(V\ C_1\ C_2\ u\ v\ \delta)$ is given by Teorell 1959 b Eq 6 Although the derivation above does not include a possible fixed charge in the membrane it is to be expected that the presence of such a charge would not affect the equations provided the pores are wide enough to make the surface conductance negligible (the fixed charge would however introduce an electroosmotic contribution to V) Accordingly the equations (1) through (4) are valid whether the water streaming V is induced by an hydrostatic and/or an (osmotic) electroosmotic pressure difference across the membrane

The integrated flux formula (3) above is convenient to use also in the presence of an externally applied electric current flow across a membrane as for instance in the above mentioned membrane oscillator because the quantities C_1 and C_2 (the salt concentrations of compartment 1 and 2) ($E_1\ E_2$) (the transmembrane potential) V (the water flow velocity) and R^{∞}_{tot} (the transmembrane resistance) are all measurable in an experiment or can be available in an analog computation procedure (cf the application on voltage clamp (Teorell 1960)) Preliminary experiments seem to indicate that Eq (3) is approximately valid also for *non* steady states where the instantaneous values of E V and R were employed

Further discussion on this ion flux equation as well as some experimental tests will be deferred to a forthcoming publication

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From the Department of Pharmacology University of Copenhagen Denmark

Exchange of I^{131} Albumin in Acute Inflammatory Oedema

By

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Received 6 March 1964

Abstract

Hvidberg E H Langgå d J Schou and L Szporny *Fxcha f I -albumin in acute inflammatory oedema* Acta phys ol scand 1964 *62* 295—303 — The sh ft of protein between plasma and experimentally induced inflammatory oedema has been stud ed in mice after ntravenous i jection of rad o odinated human serum albumin Calculations were based on the d fference between the total amounts or rad oactivity in inflamed skin samples and self control samples of equal surface areas It was demonstrated that during the initial 15 m n of the inflamma tory process the oedema contained less rad oact vity per unit we ght than the plasma Subsequent to this point however the act vity increments of the nflan ed tissue could be account ed for in t rms of extravasated plasma It was furth r shown that human serum albumin in jected intravenously to m ce disappears from the plasma according to a linea function in a semilogarithmic system The d sappearance half time was 99 min in cont ol animals but 32 m n in m ce ith local ed inflammatory oedema The average plasma volume of m ce w gh ing 25 g was 1 1 ml as determined by means of rad o od nated human serum albumin

In recent years studies of experimentally induced inflammatory oedema have been carried out extensively Most of the work has been centered upon the proteins of the oedema

It has long been known that transudation of plasma proteins across the capillary wall is almost negligible in normal tissue but greatly increased in inflamed areas (Ramsdell 1928) This phenomenon was first studied by means of different dyes bound to plasma proteins (Landis *et al* 1932 Menkin 1956 Spector 1958) but no quantitative information was yielded by these methods The use of artificial collo ds such as colloid silver (Jancsó 1961) and radioactive colloid gold (Szporny and Ezer 1962 Ezer and Szporny 1962 was later appl ed for quantitative investigations on the colloid extra vasation Réd i (1961 and Ascheim and Zweifach (1961 and 1962) used radioiodinated human serum albumin for the determination of the relative movements of protein and water across the capillary wall

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Recently a method for determining quantitatively the various constituents of the oedema fluid during acute inflammation was reported from this laboratory (Szporny Langgård and Hvidberg 1964) In the present study the same procedure was applied to the quantitative determination of the exchange of plasma proteins and water during the different phases of the acute inflammatory process For this purpose the method was supplemented with the intravenous injection of I^{131} labeled human serum albumin

The primary purpose of this investigation has thus been to determine the concentration of albumin in the oedema fluid at various times of the inflammatory process in relation to the concentration in plasma The introduction of human serum albumin however required the determination of the rate of the gradual decline in the plasma activity due to the removal of the foreign protein by the reticulo-endothelial system of the animal The present paper therefore has the additional purposes of determining the rate of removal of human serum albumin from the mouse plasma and of describing a method for the determination of the plasma volume of mice with use of I^{131} labeled human serum albumin

Methods

The white male mice used in the experiments were from a single strain weighing from 22 to 28 g They were maintained on a standard laboratory diet with water ad libitum

Initial experiments were designed to determine the rate of removal of human serum albumin from mouse plasma after its i v injection to normal mice Fifty µl of a 5 [illegible] albumin solution containing approximately 0 5 µC was injected by means of a micrometer syringe (Agla) into a tailvein Blood was sampled in heparinized capillary tubes from the tailvein opposite to the injected vein 5 and 20 min after the injection and from a neck vein 1 hour after the injection After centrifugation 15 µl of plasma was pipetted and diluted with 5 ml of water The samples were assayed in a well type crystal connected with an Isotope Developments LTD scaler 1700 After correction for background the figures were plotted in a semilogarithmic system as a function of the time and the disappearance half times were read from the curves obtained Knowing the amount of activity injected the plasma volumes were calculated from the activity per unit volume at the time of injection as determined by extrapolation back to the time of injection

For the purpose of determining the radioactivity per unit weight of the oedema fluid animals were injected as described above and the site of puncture sealed carefully To ensure uniform distribution 5 min were allowed to elapse before an initial blood sample was drawn from a tail vein The acute inflammatory process was induced as described in a previous report (Szporny Langgard and Hvidberg 1964) Briefly the procedure is that the back of the animal is depilated on the day prior to the experiment with a depilatory containing barium sulphide with the animal under light fluothane (halothane) anaesthesia two symmetric areas are marked out on the skin Acute inflammation is provoked within the borders of one of the areas by applying pieces of filter paper impregnated with xylene for 2 min with the contra lateral area serving as a control One min 5 min 15 min or 2 hours after the treatment a second blood sample was taken from the neck the animal was decapitated and bled and two skin samples with underlying subcutaneous tissue excised according to the stamping The weight of the oedema was determined as the weight difference between the two samples (w — w) The skin samples were hydrolyzed with 5 ml of 2 N potassium hydroxide and the total radioactivities of the oedematous sample (cp) and of the control sample (cps) determined The radioactivity per unit weight of oedema (cps oedema) was then calculated by the formula $\text{cps oedema} = \frac{\text{cps} - \text{cp}}{w - w}$

Plasma activities were determined as described above

The two plasma activity values determined the slope of the disappearance curve and the disappearance half time was read directly from this curve Plasma volumes were calculated as previously described In order to determine by which organs the foreign protein was taken up animals with localized inflammatory oedema and control animals were killed 1 hour after

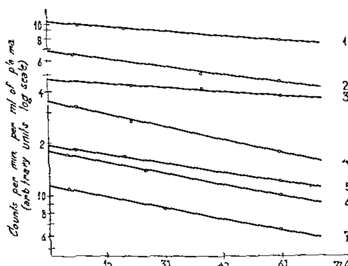


Fig 1 Radioactivity per unit volume of plasma at three different times after intravenous injection of I labeled human serum albumin to seven control animals (ordinate logarithmic scale)

TABLE I The disappearance half times for human serum albumin and the plasma volumes of mice with experimentally induced localized inflammatory oedema and of control mice after intravenous injection of I labeled human serum albumin

	Disappearance half time (min) Mean ± S E	Plasma volume (ml) Mean ± S E
Animals with localized oedema (n = 16)	32 ± 1 6	1 03 ± 0 046
Control animals (n = 7)	99 ± 15 6	1 14 ± 0 068

njection of I albumin and specimens were excised from liver spleen and spine Radioactivity of these specimens was measured after alkaline hydrolyses and the activities per unit weight compared The radioactivity of the tails was also measured to ascertain that no labeled albumin was left at the site of injection

To obtain further data on the late exchange of protein in the inflammatory oedema experiments were carried out where the oedema was induced first and the I albumin injected 15 or 10 min later These animals were killed 15 min after the injection Other procedures were the same as described above

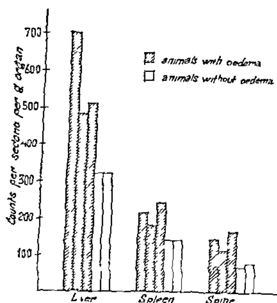


Fig 2 Radioactivity per unit eight of liver spleen and spine of three edematous and two non-oedematous mice killed and exsanguinated 1 hr after intravenous in ection of I labeled human serum albumin Factors of correction have been used to give the sam act vity per unit weight of plasma in the five animals

TABLE II The ratio $\frac{\text{counts/min/gram oedema}}{\text{counts/min/gram plasma}}$ *at different times of the oedema formation*

	Duration of oedema formation	counts/min/gram oedema / counts/min/gram plasma Mean ± S E
(n 4)	1 min	0 34
(n 10)	5 min	0 83 ± 0 065
(n − 12)	15 min	1 00 ± 0 077
(n = 9)	2 hours	1 19 ± 0 094

Results

The values obtained from seven mice in which the plasma activity was determined at three different intervals after intravenous injection of I^{131} albumin are shown in Fig 1 These figures demon trate that the foreign protein was removed from the plasma according to a linear function in a semilogarithmic system

The disappearance half time as read by the disappearance curves and the plasma volumes as calculated from the activity at the time of injection from 16 animals with localized inflammatory oedema and 7 control animals are shown in Table I The statistical computations are based on the t test and mean values are given together with the standard error of the mean (S E) Fifty of the injected activity was removed in

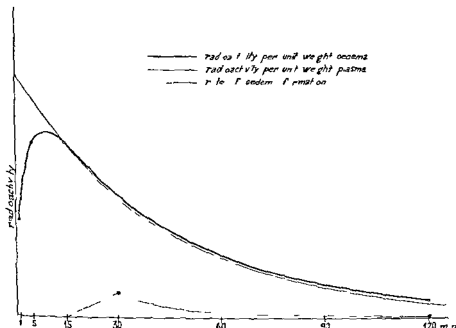


Fig 3 Schematic illustration of oedema activity and plasma activity in relation to the time
The thin line represents the aver ge radioactiv ty disappearance curve of plasma The oedema curve is drawn on basis of the plasma curve and the rat os of table 2
In luded is a third curve (dotted) based on the oedema activity obtained dur ng the 1st 2nd and 8th 15 min intervals Arb trarily this curve outlines the rate of oedema formation
(Note the ord nate radioact vity on an arithmetric scale)

99 min in control animals but in 32 min in animals with oedema The plasma volume was 1 1 ml in both groups or 4 4 % of the body weight

Fig 2 shows the activity per unit weight of various organs from 3 mice with oedema and two control mice killed and exsanguinated 1 hour after injection of I^{131} albumin In oedematous mice the activity was taken up by liver spleen and bone marrow to a greater extent than in animals without oedema

In Table II are shown the values summarizing the results of the experiment in which I^{131} albumin was inj cted 5 min before the inflammatory process was initiated and the animals killed at various hours of the oedema formation The ratio of the radioactivity per gram oedema to that of the plasma was 0 3 1 min after the treatment 0 8 5 min after the treatment and reached 1 0 at 15 min Tv o hours after the treatment the ratio was 1.2 The relationship between the oedema activity and the plasma activity during the formation of the oedema is illustrated in Fig 3 where the activities measured are plotted in an arithmetric system as a function of the time In the same figure the results obtained on two groups of animals injected 15 min (ratio = 1 19 S E 0 022) and 105 min (ratio = 0 046 S E = 0 0010) after initiation of the nflammatory process and killed 15 min later are presented

The experiments carried out 1 min after treatment with xylene presented the problem that the oedema formation at this time was very limited and the inaccuracy of

TABLE III The ratio $\frac{\text{counts min/gram oedema}}{\text{counts min gram plasma}}$ 1 min after xylene application in relation to the size of the oedema

	Size of oedema			
No	Absolute (mg)	In % (w/w) of the original tissue weight	Counts min gram oedema / Counts/min/gram plasma	Range of the ratio with a ± 5 % error on the oedema weight
1	95	34 5	0 517	0 45 – 0 59
2	50	12 0	0 215	0 13 – 0 0
3	50	11 4	0 134	0 07 – 0 [illegible]0
4	18	8.8	0 505	0 20 – 0 80
5	12	3 8	0 945	
6	10	2 7	0 258	
7	No oedema	—	+ 4 0 cps. on treated side (21 6 — 17 6)	
8	No oedema	—	+ 5 8 cps. on treated side (23 9 — 18 1)	
9	No oedema	—	+ 3 4 cps on treated side (16 3 — 12 9)	

method therefore magnified For that reason the figures of all animals studied at this time are presented in Table III Only the 4 animals with more than 5 % of oedema (i e 5 % of the original tissue weight) have been included in the calculations on which Fig 3 is based. The figures in the last column demonstrate that a 5 % error in the determination of the weight of the oedema would not have affected the impression that the relative activity of the oedema at this time is low

It is noteworthy that in twelve animals on which no oedema had developed at the time of killing the activity was always higher on the treated side

Discussion

Radioiodinated human serum albumin has been found by several investigators to serve as a reliable index of protein movements in animals Aschheim and Zweifach (1961 and 1962) have given a kinetic description of the initial phases of the inflammatory reaction by means of I^{131} labeled human serum albumin. One important aspect however seems to have been neglected in some instances Being a foreign protein, human serum albumin injected into an animal may be removed from the plasma with such rapidity that even short term experiments are critically influenced by the phenomenon

In the present study it was demonstrated that human serum albumin was removed from the mouse plasma according to a linear function in a semilogarithmic system (Fig 1) Fig 2 illustrates the fact that the activity was taken up by organs belonging to the reticuloendothelial system of the animal and that the rate of removal was con

siderably increased during the inflammatory process (see also table 1) supporting the view that an activation of the reticuloendothelial system constitutes part of the inflammatory response (Jancsó 1961) The rate of removal is only in little extent influenced by the escape of plasma into the oedema since the plasma activity depends on the albumin *concentration*

The plasma volumes reported using the present method (Table I) were not the major aim of the study They have however been included because the values confirm the reliability of the technique

A second advent of the present study is the application of a method to quantitating the intensity and progression of the inflammatory reaction (Szporny *et al* 1964) The term oedema refers to the weight difference between an inflamed skin sample and a corresponding control sample *i e* to the plasma filtrate itself and not to the mixture which the plasma filtrate forms with preexisting extracellular fluid This concept of the oedema allows for exact quantitative calculations on the *genuine oedema in vivo* and *in situ* (*i e* within the connective tissue)

In the present study the ratio between counts per minute per gram oedema and counts per minute per gram plasma has been used as a measure of the protein extravasation This ratio should be equal to 1 0 if the oedema was due only to an extravascular accumulation of plasma

It was found that during the initial 15 min after xylene application the oedema contained less radioactivity per unit weight than the plasma Subsequent to this phase however the activity increments on the treated side could be accounted for in terms of extravasated plasma (Table I and II and Fig 3)

Ascheim and Zweifach (1961 and 1962) demonstrated the existence of three distinct phases of the early inflammatory oedema formation an immediate reaction (0—3 min) where the water filtration exceeded the protein leakage a second phase (3—8 min) during which the protein accumulation transiently exceeded the water filtration and a third protracted phase when the oedema was accountable in terms of extravasated plasma As stated above only two phases could be demonstrated in the present study The first phase corresponded well to the initial phase described by the above mentioned authors but lasted for 15 min and proceeded directly into the second phase which corresponded to their third phase An intermediate phase with retention of proteins could not be demonstrated

The discrepancy perhaps is due to the different techniques and the different modes of calculation

The 1 0 ratio found 15 min after application of xylene is in good accordance with the statement of Szporny *et al* (1964) that the oedema at this stage of the inflammatory process is a bulk filtrate of plasma The figure indicates that during the initial phase of the oedema formation 15 min is sufficient time for a total equilibrium of plasma proteins The connective tissue ground substance may represent a barrier permitting small molecules to move faster than the proteins This would explain the low ratio initially even if *the capillary wall were equally permeable to all plasma components.*

In the above experiment the radioactive albumin was circulating before provocation of oedema If however the albumin was injected 15 min or 105 min after the xylene treatment only 20 and 4 respectively of the plasma activity was obtained by the oedema during the 15 min period following injection

In Fig 3 it has been attempted to illustrate arbitrarily the rate of oedema formation on basis of these findings (dotted line)

The results are in agreement with a previous report (Langgård Szporny and Hvidberg 1964) carried out under the same experimental conditions as were used in the present study when it was found that 50 % of the oedema was formed within the first 15 min after xylene application but that the volume of the oedema continued to increase for 2—3 hours

The 1 2 ratio two hours after application of xylene can be reasonably explained if the rate of exchange with the plasma at this time is exceeded by the rate of removal of radioactivity from the plasma

The findings are also in agreement with the data obtained by Szporny and Ezer (1962) and Ezer and Szporny (1962) who after injection of Au labeled colloid gold found that the ratio between the activity of the inflamed and the untreated paw of rats rose only during the first 15 min although the oedema continued to develop for a much longer period of time

In several animals (1 and 5 min expts) no oedema had developed at the time of sacrificing the animals In these cases it was a constant finding that the activity was slightly increased on the treated side Since the same phenomenon could not be demonstrated after intravenous injection of C labeled glucose (unpublished data) this rise of activity which preceeds the extravasation of plasma could not be explained by capillary dilatation Jancsó (1961) has shown the formation of fibrin films with different colloids attached to them along the endothel of capillaries and microclots of small vessels in the initial phase of inflammation This phenomenon which he named endothel activation regularly preceds the oedema formation The early protein accumulation found in the present study may be a manifestation of this endothel activation

In conclusion the results obtained indicate that from the fifteenth min of the oedema formation the proteins of the inflammatory oedema and the plasma are in a state of dynamic balance as estimated from the radioactivity measurements This equilibrium is maintained in spite of the rapid removal of radioactivity from the plasma This in turn is an indication of the rapidity of the process of reabsorption of proteins from the oedema

It is generally agreed that in normal tissues colloidal substances are absorbed from the tissues *via* the lymphatics A very rapid reabsorption of proteins could thus be brought about by an increased lymph drainage or by the capillary wall becoming transiently permeable to proteins in both directions Rusznyak Foldy and Szabo (1957) have demonstrated a considerable rise in the lymph drainage from inflamed areas Menkin (1956) on the other hand found an obstruction of the lymphatic endings and a tissue fixation of colloids at the place of inflammation Szporny and Ezer (1961) in rat paw oedema and Langgård (1963) in patients with thrombophlebitis found an enhanced absorption of colloids but a diminished absorption of crystalloids These findings seem to indicate that the dynamic balance between the proteins of inflammatory oedema and plasma is maintained by means of the lymphdrainage

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Acta physiol scand 1964 62 304–312

From the Neurophysiological Laboratory Anatomical Institute University of Oslo Norway

Uterine Motility of the Estrogenized Rabbit

IV Reflex Excitation and Inhibition

By

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Abstract

Setekleiv J *Uterine motility of the estrogenized rabbit IV Reflex excitation and inhibition* Acta physiol scand 1964 62 304–312 — The uterus can be reflexly activated by stimulation of the rectum various somatic nerves and the central and peripheral ends of the cut vagus nerve The initial uterine contraction is followed by a period of reduced rhythmic activity Similar responses can be elicited by asphyxia and by clamping of the common carotid arteries All these responses are due to activation of the sympathico-adrenal system A transient inhibition of the spontaneous rhythmic activity is obtained by electrical stimulation of the depressor nerve Since it is abolished by sympathetic blocking agents it is most likely due to a reduction of a sympathetic tonic influence The results indicate that although the autonomy of the myogenic rhythmic uterine activity is strong in the estrogenized rabbit it can be influenced by various reflexes It is suggested that the sympathetic nervous system exerts a tonic influence on the uterus *in vivo*

Most studies on reflex excitation of the uterus have been carried out from about fifty to a hundred years ago Kurdinowsky (1907) observed uterine contractions in response to stimuli applied to the mamma skin stomach and intestine Uterine contractions have also been recorded on stimulation of various peripheral somatic nerves (Schlesinger 1873 Falk 1913) Furthermore uterine contractions have been elicited by stimulation of the peripheral end of the cut vagus nerve (Kilian 1851 Kehrer 1867 Falk 1913) as well as of its central end (Kilian 1851) The latter effect could not be confirmed by Obernier (1862) Spiegelberg (1864) and Frankenhauser (1866)

Later uterine contractions in response to mechanical stimulation of pelvic viscera were described by Ferguson (1941) who observed contractions of the uterus after irritation of the cervix vagina and rectum. These findings were in part confirmed by Cross (1958)

In the present study the reflex excitation of the uterus to stimulations of the rectum somatic nerves and the central and peripheral cut end of vagus have been confirmed



Fig 1 A Uterine response to mechanical stimulation of the rectum Curarization of th an m l abolished the accompanying abdominal contractions but d d not bl ck the uterin response (B) whereas spinal anesthesia abol shed the reflex (C) (Exp 69)

The effects were found to be due to an activation of the sympathico-adrenal system Further some other procedures known to activate this system such as asphyxia or oc clu ion of the carotid arteries were found to stimulate the uterus in a similar way

To the author s knowledge reflex inhibition of the uterine motility has not been previously reported In the present study an inhibition of uterine motility was obtained by stimulating the depressor nerve and various other cranial nerves

Material and methods

Thirty seven spayed estrogenized rabb ts (weights from 2 5 to 3 3 kg) were used Ovariectomy was perf rmed at least one week bef re the experiment and the anim als then treated with 0 1 per cent d ethylstilbestrol 0 5 ml every second day

The *operative p ocedu es* and th e *re rding te hnique* have been descr bed in prev ous comm un ca t ns (Set kle v 19( ) a and b The rabbits were anesthetized w th a mixture of chloralose (1 per cent) and urethane (?5 per cent) 40 and 500 mg kg respectively one half adm nistered v and the rest m

B polar st mulat on of the n rves was made by square wave pul es intens t es from ? to 8 V (0 3? – 1 ?8 mA) 1 msec pulse durat or and frequenc es from 5 to 100 cps El m nation of abdom nal muscular contractions was secured by immobilizat on w th succ nylchol ne (Curas t Nyco) and art fic al respiration

A phyxia was produ ed by clamping of th tra heal cannula *occlusi of the carotid arterie* by a forceps w th rubber-coated branches

Results

A *Reflex excitation of the uterus*

1 *Rectal stimulation* The first part of the experiment was conducted under light anesth sia only Some manipulations of the thermometer used for read ngs of th b dy t mpera ture were used as ar adequate mechanical stimulat n of the rect m in three animals The uterine resp nse cons sted of an increased contraction foll wed by an inh b t on of the spontaneous rhythmic a t v ty Fig 1 A The latency of the resp nse was about ?0 sec and under the present condit ons t was accompanied by ontractions of the abdominal muscles The an mal was ther paral zed with succinylcholine and artif cially entilation started Th uterine response to rectal stimulation was thereby somew t at

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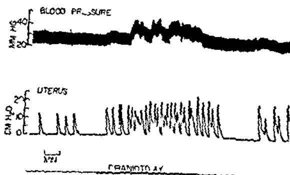


Fig 2 Effects on blood pressure and uterine motility of painful stimuli (craniotomy) (Exp 108)



Fig 3 Uterine response to electrical stimulation of the central end of a cut branch of the brachial plexus (3 V 50 cps 1 msec) (Exp 59)

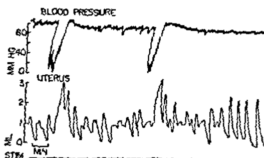


Fig 4 Uterine response to electrical stimulation of the peripheral end of the cut cervical vagus (5 V 50 cps 1 msec) (Exp 71)

reduced (Fig 1 B) But after spinal anesthesia was achieved by instillation of 1 *ml* (2 per cent) lidocaine (Xylocain® Astra) intrathecally the response to rectal stimulation was abolished (Fig 1 C) That indicates that the response was mediated by a spinal reflex

2 *Stimulation of somatic nerves* In lightly anesthetized animals operative procedures such as craniotomy produced an increased uterine activity and elevation of blood pressure (Fig 2) Likewise electrical stimulation of the central end of the cut sciatic and femoral nerves and branches in the brachial plexus elicited a uterine contraction within 7—10 sec (Fig 3) The contraction was followed by an inhibition of the rhythmic activity and resembled the response to adrenaline and noradrenaline Adrenalectomy did not alter the response whereas additional section of the hypogastric nerve abolished it

3 *Vagal nerve stimulation* In 6 out of 16 animals electrical stimulation of the *peripheral cut end* of the cervical vagus resulted in a contraction of the uterus followed by an inhibition of its rhythmic activity (Fig 4) The latency of the response was 10—20 sec

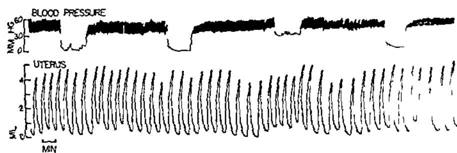


Fig 5 No effect on uterine activity by occlusion of the abdominal aorta as indicated by the signal line below Blood pressure recorded from the femoral artery (Exp 60)

and the resemblance of the response to the effect on the uterus of adrenaline administration suggests an activation of the sympathico-adrenal system The effect of peripheral vagal stimulation persisted after adrenalectomy whereas sectioning of the hypogastric nerve reduced the response and prolonged its latency

The uterine response to peripheral vagal stimulation was accompanied by a marked fall in blood pressure (Fig 4) In order to determine whether a similar drop in blood pressure *per se* had any influence on the uterine motility a local blood pressure fall was produced by clamping of the abdominal aorta for up to two minutes. This procedure had little or no effect on the rhythmic uterine activity (Fig 5) A prolonged ischemia of several minutes duration was required to influence the amplitude and frequency of the rhythmic contractions The initial effect of such prolonged ischemia was a reduction in amplitude of the rhythmic contractions accompanied by an incomplete relaxation between each contraction

It is concluded that the influence on uterine contractions of the stimulation of the distal end of the cut vagus is not secondary to the induced blood pressure fall but most likely to reflex activation of the sympathico-adrenal system

Electrical stimulation of the *central* end of the cut vagus nerve produced after a latency of few seconds a rise in blood pressure followed by a uterine response similar to that which follows an activation of the sympathico-adrenal system Thus, in lightly anesthetized animals merely touching the vagus nerve with the electrode induced a clearcut response

The response could be elicited after removal of the hypothalamus by suction (Fig 6 A) indicating that the reflex center is located at a lower level

4 *Asphyxia* Closing of the tracheal cannula resulted in increased activity of the uterus after a latency of 25—45 sec calculated from the onset of the clamping of the cannula Usually the response consisted of one or a few contractions of increasing amplitude and incomplete relaxation between successive contractions (Fig 6 B) At the end of the period of asphyxia the rhythmic activity diminished for a period of several minutes The blood pressure increased during asphyxia The uterine response to asphyxia was neither abolished by bilateral adrenalectomy nor by section of the hypogastric nerve However the response was delayed after the latter procedure

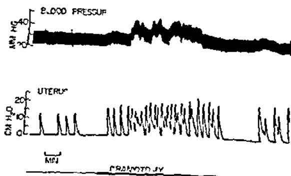


Fig 2 Effects on blood pressure and uterine motility of painful stimuli (craniotomy) (Exp 108)

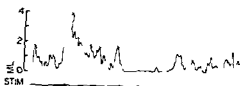


Fig 3 Uterine response to electrical stimulation of the central end of a cut branch of the brachial plexus (3 V 50 cps 1 msec) (Exp 59)

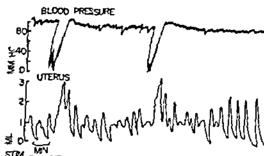


Fig 4 Uterine response to electrical stimulation of the peripheral end of the cut cervical vagus (5 V 50 cps 1 msec) (Exp 71)

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2 *Stimulation of somatic nerves* In lightly anesthetized animals operative procedures such as craniotomy produced an increased uterine activity and elevation of blood pressure (Fig 2) Likewise *electrical stimulation of the central end of the cut sciatic* and femoral nerves and branches in the brachial plexus elicited a uterine contraction within 7—10 sec (Fig 3) The contraction was followed by an inhibition of the rhythmic activity and resembled the response to adrenaline and noradrenaline Adrenalectomy did not alter the response whereas additional section of the hypogastric nerve abolished it

3 *Vagal nerve stimulation* In 6 out of 16 animals electrical stimulation of the *peripheral* cut end of the cervical vagus resulted in a contraction of the uterus followed by an inhibition of its rhythmic activity (Fig 4) The latency of the response was 10—20 sec

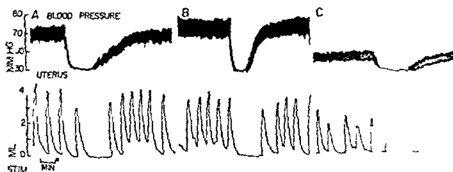


Fig 7 *A* Effect of depressor nerve stimulation (6 V 50 cps 1 msec) on blood pressure and uterine rhythmic activity *B* Atropine (0 54 mg/kg) did not alter the uterine response whereas it was abolished after 0 54 mg/kg of dihydroergotamine (*C*) (Exp 90)

ear vein (trigeminal nerve) as well as by manipulation of the external auditory canal (innervated by the glossopharyngeal nerve) Occasionally similar but weaker and short lasting inhibition could be elicited by mechanical stimulation of the nostrils

Discussion

A *Reflex excitation of the uterus*

1 *Rectal stimulation* The findings of Ferguson (1941) on reflex excitation of the uterus by mechanical stimulation of the rectum has been confirmed and also the spinal mediation of the reflex (Cross 1958) Since high spinal transection does not abolish the responses (Cross 1958) the reflex does not require the participation of supraspinal structures The spinal reflex are contains both a somatic and a visceral limb In the present investigation, curarization of the animal abolished the abdominal contractions but not the uterine response

Uterine contractions elicited reflexly from pelvic viscera are believed to play an important role in labour (Cross 1959) The stimulus may be movements of the foetus or dilatation of the lower uterine segment cervix and vagina during passage of the foetus The effect of enema in obstetrics to induce labour may partly be due to reflex influence on the uterus from the rectum

2 *Stimulation of somatic nerves* elicits a reflex uterine response through the sympathico-adrenal system Most likely this reflex is produced by stimulation of pain fibres An increased adrenal medullary discharge has previously been found after such stimulation (Cannon and Rapport 1921 Euler and Folkow 1953

3 *Vagal nerve stimulation* The uterine response to stimulation of the *peripheral* end of the cut vagus resembles the uterine response to administration of adrenaline Since it can be elicited after adrenalectomy the response cannot be caused by direct activation of the adrenal medulla Most likely it is due to activation of the sympathetic nervous system by the blood pressure decrease acting on the baroreceptors In favour of this view stand the findings of Gernandt Liljestrand and Zotterman 1945 who recorded increased spike activity in the splanchnic nerve during low blood pressure

The excitatory influence on the uterus of the stimulation of the *central* end of the cut vagus confirms the observation of Kilian more than a century ago (1851) It was also

confirmed that such stimulation produces an increased blood pressure (Neuman 1914 Cragg and Evans 1960) These observations point to an activation of the sympathico-adrenal system which is in agreement with other responses to central vagal stimulation such as increased discharge from the adrenal medulla (Cannon and Rapport 1921 Houssay and Molinelli 1927) and gastric relaxation (Cragg and Evans 1960)

It has been suggested that the pressor response after stimulation of the central cut end of the vagus is due to a reflex release of post pituitary hormones (Chang *et al* 1938) In the present study neither the pressor nor the uterine responses were abolished after removal of the hypothalamus Similar results have been obtained by Taylor and Page (1952) with respect to the pressor response in the dog The hypothalamus is therefore not essential for mediation of the response The afferent impulses most probably excite structures in the brain stem activating the sympathetic nervous system and thereby the uterus

4 *Asphyxia results in a uterine response characteristic of the activation of the sympathico-adrenal system* Induction of uterine contractions by asphyxia (cessation of respiratory movements) was already observed by Spiegelberg (1864) and Schlesinger (1873) By comparing with responses of various sympathetic effector organs and by measuring the adrenal medullary output asphyxia has been determined to be the most powerful physiological stimulus of the sympathico-adrenal system (for ref see Celander 1954)

5 *Clamping of both carotid arteries* was found to induce uterine contractions Here again the mechanism is probably an activation of the sympathico adrenal system by means of the baroreceptors responding to the blood pressure fall and the chemoreceptors to the reduce blood supply Carotid occlusion has previously been shown to induce sympathetic responses from other effector organs such as the nictitating membrane the spleen and the intestines (Celander 1954 Kock 1959)

The findings reported here have demonstrated that activation of the uterus may be elicited by pain asphyxia and disturbance of the carotid blood flow This should be taken into account in experiments on the uterus in the intact animal Whether this mechanism is activated during labour pain cannot be answered at our present state of knowledge

The effects of hypogastric nerve section and of adrenalectomy suggest that the stimulation of the uterus is mediated through both components of the sympathico-adrenal system Because of the variability of the response it has not been possible to perform a quantitative estimation of the relative role played by each of these two routes Sympathetic stimulation of blood vessels the nictitating membrane and the spleen has been found to be mainly due to stimulation through its nervous supply The adrenal medullary discharge is postulated to play an accessory role only (Celander 1954, On the other hand the reflexly induced inhibition of intestinal motility has been found to be practically exclusively dependent on the hormonal component (Kock 1959)

B *Reflex inhibition of the uterus*

1 *Depressor nerve stimulation* It is assumed that the aortic or depressor nerve originates from the aorta and the subclavian artery (Tello 1924 Nonidez 1935) although the possibility that it contains in addition fibres from other thoracic structures has been discussed (Douglas Ritchie and Schaumann 1936) In rabbits electrical stimulation of this nerve produced depressor responses only whereas in cat pressor responses were also elicited at certain intensities (Douglas and Schaumann 19_6)

The present observation that the uterus can be inhibited by depressor nerve stimulation adds another example to the rather widespread inhibitory influence exerted by this nerve on blood pressure respiration muscular tone and the knee jerk (Heymans and Neil 1958)

The uterine inhibition obtained by stimulation of the depressor nerve cannot be due solely to the concomitant fall in blood pressure because occlusion of the abdominal aorta for a limited period or stimulation of the peripheral end of the cut vagus nerve does not similarly reduce the rhythmic activity

It has not been possible to inhibit the uterus by stimulation of its peripheral nervous supply However that the uterus *in situ* is exposed to inhibitory influences was observed several times after deepening the anesthesia This increased the rhythmic activity indicating a release of inhibition (Setekleiv 1964 a) The anesthetics per se had only a depressive action on the myometrium

The vasomotor effects of depressor nerve stimulation have been found to be mediated by a medullary pathway most likely by activation of the inhibitory reticulo-spinal system (Heymans and Neil 1958) Centrally induced vasodilatation may be caused in two ways either by the cortico-hypothalamo-spinal system first described by Eliasson *et al* (1951) or by lowering of the sympathetic tone on the vessels (Folkow Johansson and Oberg 1959) Since the response was found to be mediated through adrenergic nerves it is probable that the inhibition of the uterine motility is produced by the latter mechanism

2 *Other cranial nerves* The present investigation suggests that a reflex inhibitory influence on the uterus can be elicited by stimulation of other cranial nerves such as the trigeminal and the glossopharyngeal nerves Reflex inhibition of skeletal muscles to stimulation of olfactory trigeminal and glossopharyngeal nerves has been described by Allen (1929) Frankenhaeuser and Lundervold (1949) and Andersen (1953) These reflexes possibly participate in an arrest reaction to noxious stimuli or a playing dead reaction

In investigation with estrogenized rabbit one is at first struck by the strong autonomy of the myogenic rhythmic contractions However the present investigation has shown that this myogenic activity can be influenced by a number of reflexes excitatory as well as inhibitory Furthermore the sympathetic nervous system seems to exert a tonic influence upon the uterus in situ Since this influence is susceptible to anesthesia it is likely that the nervous control of the uterus is more prominent in the absence of anesthesia

Sponsored in part by the A[illegible] F[illegible]ce Off[illegible]e of Sc[illegible]entific Research OAR thr[illegible]gh the Eur[illegible]pean Offi[illegible]e Aerospa[illegible]e Resear[illegible]h United States Air Force (Grant AF EOAR 62 36) and in part by th[illegible] Norwegian Resea[illegible]ch Counc[illegible] f[illegible]r Sc[illegible]ence a[illegible] Human[illegible]

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Acta physiol scand 1964 62 313—322

From the Neurophysiological Laboratory Anatomical Institute University of Oslo Norway

Uterine Motility of the Estrogenized Rabbit

V Response to Brain Stimulation

By

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Received 16 March 1964

Abstract

Setekleiv J *Uterine motility of the estrogenized rabbit V Response to brain stimulation* Acta physiol scand 1964 62 313—322 — Uterine contractions were elicited by electrical stimulation of points located in the lateral part of the medulla oblongata whereas inhibitory responses were induced from the midline medullary region Cerebellar stimulation determined an inhibition of the rhythmic uterine activity followed by a rebound effect, on cessation of the stimulation From the hypothalamus three different responses were obtained (i) excitatory effects from the perifornical the dorsal lateral and posterior hypothalamic areas due to sympathetic activation (ii) uterine excitation by oxytocin liberation induced from the ventromedial part of the hypothalamus and (iii) inhibition of the uterine rhythmic activity by stimulation of a restricted region in the lateral hypothalamic area. Excitatory responses were also evoked from the amygdala from sensorimotor cortex, and the anterior limbic region Inhibitory uterine responses were produced from the two cortical areas just mentioned as well as from the orbital surface

Influence of cerebral structures on the uterine motility was first reported by Budge (1841) who observed movements of the uterus after stimulation of the cerebellum and the medulla oblongata These findings were confirmed by Kilian (1848) Spiegelberg (1858) and Korner (1865)

A new era regarding the influence of brain stimulation on the uterus was opened by the investigation of Haterius and Ferguson (1938) who obtained uterine contractions due to liberation of oxytocin as a result of stimulating the infundibular stalk of the hypophysis in the post partum rabbit These findings were later confirmed by Harris (1947) Kurotsu *et al* (1952) reported that stimulation of various regions of the hypothalamus could induce uterine contractions Cross (1958) found that the uterine responses to hypothalamic stimulation were either mediated through the sympathetic nervous system or by oxytocin secretion and determined the hypothalamic areas concerned in these responses

With regard to telencephalic structures Inguilla and Principe (1950) obtained uterine contractions in cats by stimulation of the precruciate gyrus In dogs and rabbits Koike

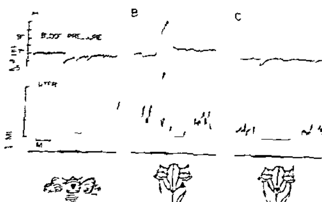


Fig 1 *A* Decrease in blood pressure and inhibition of uterine rhythmic activity followed by a rebound effect by electrical stimulation of the cerebellum (3 V 50 cps 1 msec) *B* Blood pressor response and uterine contraction followed by inhibition of the rhythmic contraction obtained by stimulation of the lateral part of medulla oblongata whereas a fall in blood pressure and inhibition of uterin activity was produced by stimulation near the midline 1.5 V 50 cps 1 msec) Latency of excitatory response 3 sec of inhibitory responses 15 sec (Exp 77

gami, Yamada and Usui 1954 elicited similar responses by stimulation of the amygdaloid complex and Beyer Anguiano and Mena 1961 described uterine contractions in cats by stimulation of the anterior cingulate gyrus

In the present investigation the brain of the estrogenized rabbit has been explored by electrical stimulation with the purpose of localizing the various regions capable of influencing the uterus In addition to excitatory responses described by previous investigators inhibition of the rhythmic uterine activity was also demonstrated

Material and methods

The results are based on data gained from 41 rabbits (2.4—3.3 kg b w The animals were spayed and treated with 0 mg diethylstilbestrol (0 1 per cent every second day for at least one week

The operative procedures and the recording techniques were the same as reported elsewhere Setekleiv 1964 a

Anesthesia The animals were anesthetized with 40 mg kg chloralose (1 per cent and 500 mg/kg urethane 25 per cent Either or small doses of pentobarbital sodium Nembutal Abbott were given in addition during the operative procedure if necessary Spinal anesthesia immobilization by succinylcholin (Curasit Novo and artificial respiration were carried out as described previously Setekleiv 1964 a

Electrical stimulation of the brain After craniotomy the cerebral and cerebellar surfaces were stimulated through bipolar electrodes with a distance of about 1—2 mm between tips In some animals one eye was enucleated in order to reach the orbital surface of the hemisphere Cooling of the cerebral cortex was prevented by high room temperature and by covering the surface with warm mineral oil which had at the same time a beneficial effect against drying Intracerebral structures were stimulated through bipolar electrodes 0 7 mm thick insulated except for one mm at the tip The head was fixed in a Horsley-Clarke frame and the electrode oriented according to the coordinates published by Sawyer Everett and Green 1954

Square pulses of 1 msec duration and at frequencies from 1 to 500 cps were employed When not stated otherwise a frequency of 50 cps was used The intensity varied from 1 to volts 0 16—0 8 mA)

Points which produced positive responses on stimulation were marked electrolytically At the termination of the experiment the brain was removed fixed in 10 per cent formalin and the electrode placements identified in thionine-stained sections 20 µ thick every 20th section stained) Intervening sections were studied when necessary

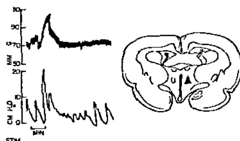


Fig 2 Sympathetic uterine response to stimulation of the periforn cal area of hypothalamus (2 V 50 cps 1 msec) Latency 3 sec (Exp 150)

Results

Medulla oblongata In five animals electrical stimulation of the lateral parts of the exposed dorsal surface of the medulla oblongata elicited a uterine response consisting of a contraction followed by inhibition of the rhythmic activity a pattern which is characteristic of sympathetic activation (Fig 1 B) The latency was 2—4 sec suggesting direct nervous activation of the uterus (Setekleiv 1964 b) The responses were elicited at relatively low stimulus intensities (1—2 V) and optimal effects were obtained at a frequency of about 50 cps There was a concomitant rise in blood pressure during stimulation

Stimulation of the medial portion of the floor of the fourth ventricle resulted in an inhibition of the rhythmic activity (Fig 1 C) The latency of the response was about 15 sec and the inhibition could be maintained 1—3 min by prolonged stimulation Escape then occurred despite uninterrupted stimulation The effect was abolished by sectioning the hypogastric nerve The uterine inhibition was associated with a fall in blood pressure a decreased muscular tone in the limb muscles and inhibition of the knee jerk

Cerebellum Stimulation of the lobus medius medianus and the lobus ansoparamedianus of the cerebellum produced in four rabbits an inhibition of the rhythmic uterine contractions lasting from one to three minutes followed by a rebound effect (Fig 1 V)

Hypothalamus The hypothalamus was stimulated in 25 animals Three different types of responses were obtained

(i) An initial contraction followed by inhibition of the rhythmic uterine activity (Fig 2) This is the uterine response typical for sympathetic activation The latency measured 2—5 sec indicating that it is mediated directly by a nervous pathway Following section of the hypogastric nerves the latency increased to about 10—15 sec and the uterine response was smaller When in add tion the adrenal glands were removed the uterine response to hypothalamic stimulation was abolished

Concomitantly with the uterine response there was a rise in blood pressure widening of the palpebral fissure retraction of the nictitating membrane and pupillary dilatation

At just above threshold stimulation (0 5—1 V) the most sensitive points were localized in the perifornical the dorsal lateral and posterior areas of the hypothalamus (Fig 3) Higher stimulation intensities elicited uterine responses from most electrode placements

(ii) An initial contraction followed by several contractions of the same or somewhat smaller amplitude The latency varied from 15 to 25 sec and no inhibitory period of the rhythmic activity was seen in this type of response which had a similar pattern a the uterine response to i v administration of oxytocin Fig 4) In the estrogenize

Fig 3 Diagram of frontal sections through the rabbit's hypothalamus (after Sawyer *et al* 1954) with stimulation points yielding excitatory (▲) inhibitory (▼) and no (●) responses Large symbols indicate the points of lowest intensities of stimulation i e lowest threshold

Abbreviations

AC Anterior commissure — AM N anteromedialis — ARC N periventricularis arcuatus — AV N anteroventralis — C N caudatus — CC Corpus callosum — CM Centrum medianum — DHA Dorsal hypothalamic area — DM N dorsomedialis — DMH N dorsomedialis hypothalami — EC External capsule — FF Fimbria of fornix — FX Fornix — GP Pallidum — HPC Hippocampus — HT Habenulo interpeduncular tract — IC Internal capsule — LA N lateralis anterior — LHA Lateral hypothalamic area — LP N lateralis posterior — LPO Lateral preoptic area — M Massa intermedia — MG Medial geniculate body — ML N mammillaris lateralis — MM N mammillaris medialis — MPO Medial preoptic area — MT Mammillo thalamic tract — NC N centralis — OCH Optic chiasma — OT Optic tract — PED Basis pedunculi — PHA Posterior hypothalamic area — PMA Premammillary area — PUT Putamen — PV N paraventricularis — RET N reticularis — SCH N suprachiasmaticus — SMA Supramammillary area — SN Substantia nigra — SO N supraopticus — SOD N supraopticus diffusus — SP Septum pellucidum — STH Subthalamus — VA N ventralis anterior part — VEN Ventricle — VL N ventralis lateral part — VMH N ventromedialis hypothalami — VML Ventral medullary lamina — VPL N ventro-postero-lateralis — VPM N ventro-postero-medialis

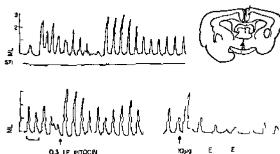


Fig 4 Oxytocin response to hypothalamic stimulation Latency ?0 sec The response consists of several uterine contractions and has a similar pattern as the uterine response to i v injection of pitocin Adrenaline given i v produces a different response The electrode track and the stimulated point in *dicated on the inset drawn from* the serial section (Exp 40)

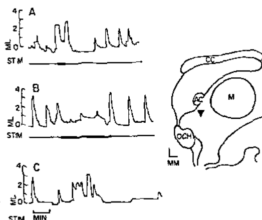


Fig 5 Inhibition of uterine rhythmic activity by electrical stimulation of the lateral hypothalamic area just beneath the anterior commissure ? mm lateral to the midline (5 V 50 cps 1 msec) (B) Stimulation 2 mm above (A) and 2 mm below this point (C) elicited uterine contractions (Exp 23)

rabbit it was difficult to obtain typical oxytocin responses because the sympathetic responses tended to dominate In one rabbit stimulated 8 hours after parturition the oxytocin response was more easily recognized (Fig 4) The effects were obtained by stimulation in the ventromedial part of hypothalamus in the vicinity of the para ventricular and supraoptic nuclei and the supraoptico-hypophyseal tract

(iii) Inhibition of the rhythmic uterine activity was obtained in three rabbits by stimulation of points localized in the lateral hypothalamic or lateral preoptic area Fig 5 shows the abrupt alteration of the uterine response when the electrode was lowered through the hypothalamus The loci responsible for inhibition were located about 2 mm lateral to the midline just below the posterior border of the anterior commissure

Amygdala The amygdaloid complex is a third region of the brain which is known to influence a number of autonomic activities In all 11 rabbits stimulated in this region an increase of uterine contractions ensued The responses were usually weak T" was a concomitant rise in blood pressure and inhibi intestinal motility corded by a balloon in the rectum) indicating a sy activation (Fig 6) latency of the uterine response measured from esting a nervous at least for the initial part of the effect The ould not

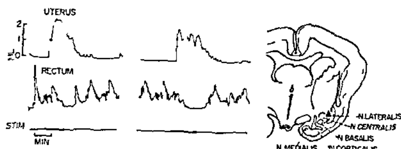


Fig 6 Excitatory uterine response to amygdaloid stimulation (5 V 50 cps 1 msec) The latency (7 sec) and the concomitant inhibitory effect on rectal response indicate sympathetic activation Right Localization of the point stimulated (Exp 38)

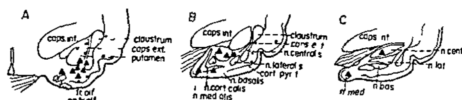


Fig 7 Frontal section through the amygdala in rabbits indicating points yielding excitatory uterine responses (▲) to electrical stimulation (●) No response

any particular subdivision of the amygdaloid complex although most of them were found in its central and medial portion (Fig 7)

The cerebral cortex The cortex of the cerebral hemispheres including most of its medial and ventral aspects was explored in 21 rabbits Both excitatory and inhibitory uterine responses were obtained The cortical stimulation points yielding excitatory responses were grouped in the sensorimotor cortex I as outlined by Woolsey (1958) (Fig 8 A) and the anterior limbic region (Fig 8 B) Inhibitory effects were induced from these regions as well as from the orbital gyrus (Fig 8 C) The responsive cortical areas are outlined in the summary diagram of Fig 9

The effects of cortical stimulation were weak compared with the uterine reaction to hypothalamic stimulation The responses appeared under light anesthesia only and were also obtainable solely in animals in good condition The latency of the response varied from 5 to 25 sec The variability and the inconstancy of the responses made it difficult to determine the peripheral pathways in section experiments However the *inhibition of the intestinal motility which occurred simultaneously suggests* that the *uterine* responses were due to sympathetic activation (Fig 8 A) Alterations of *intestinal* motility were found to be a more sensitive indicator of sympathetic activation than was the uterine motility *The intestinal and blood pressure responses to cerebral stimulation* will be dealt with in a separate communication (Setekleiv 1964 c)

The genuineness of the cortical response was demonstrated by the abolition of the response by local application of lidocaine (Xylocain© Astra) 2 per cent to the cerebral cortex *Curarization did not influence* the uterine response to cortical stimulation indicating that it is not secondary to modifications of respiratory movements or to contractions of the abdominal muscles

Fig 8 A Excitatory uterine response to electrical stimulation (5 V 50 cps 1 msec) of the sensorimotor cortex and the concomitant inhibition of the rectum (Exp 38) *B* Excitatory uterine response to electrical stimulation (4 V 50 cps 1 msec) of pregenual portion of the anterior limbic region and inhibitory response to stimulation of the subcallosal cortex (same stim para meters) (Exp 15) *C* Decrease in blood pressure and inhibition of the uterine rhythmic activity by electrical stimulation (4 V 50 cps 1 msec) of the orbital surface of the frontal lobe (Exp 147)

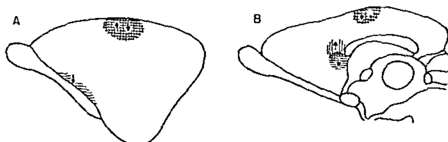


Fig 9 Regions of the lateral (A) and medial (B) aspects of the rabbit hemisphere from which excitatory ( ) (vertical hatching) and inhibitory ( ) uterine responses (horizontal hatching) were induced by electrical stimulation

Discussion

The present investigation has shown that the rabbit uterus can be influenced by cerebral stimulation in three ways (1) excitation due to sympathetic activation (2) excitation caused by oxytocin secretion and (3) inhibition of the rhythmic activity

(i) *The sympathetic excitatory response* is similar to the effects on the uterus of hypogastric nerve stimulation or i v administration of adrenaline and noradrenaline (Setekleiv 1964 b) It is most readily elicited from the lateral parts of the medulla oblongata and the hypothalamus This medullary localization appears to coincide with those regions from which other facilitatory responses have been elicited such as blood pressure increases (Alexander 1946) facilitation of cortically and reflexly evoked movements (Magoun and Rhines 1946) and contraction of the urinary bladder (Kuru Kurati and Koyama 1959)

The hypothalamic regions determined by threshold stimulation as most effective in producing sympathetic uterine responses correspond well with the findings of Cross (1958) The location of the responsive sites also corresponds well with the hypothalamic regions from which other sympathetic autonomic and behavioural responses have been produced by electrical stimulation (Ranson and Magoun 1939 Hess 1954)

The excitatory uterine responses produced by stimulation of the amygdaloid nuclear complex were weak compared to those elicited from the hypothalamus The latency and the concomitant inhibition of the intestinal motility suggest a sympathetic activation Stimulation of the amygdaloid complex has been shown to produce a number of complex behavioural as well as autonomic effects (for references see Ursin and Kaada 1960) More recently another pelvic organ the urinary bladder has been shown to be influenced by amygdaloid stimulation (Gjone 1964)

There are only few studies on the cerebral influence on autonomic activities in the rabbit whereas a large number of such studies have been carried out in cats dogs and monkeys (*cf* Kaada 1961) The cerebral cortex in the rabbit represents a lower level of evolution compared to that of the cat The present study has demonstrated that uterine contractions through sympathetic activation can be evoked by stimulation of the sensorimotor cortex and the anterior limbic region These observations are in agreement with those of Inguilla and Principe (1950) and Beyer *et al* (1961) in cats However the latter authors interpreted the response to be due to oxytocin secretion

The physiological significance of this sympathetic activation of the uterine motility induced by brain stimulation is unknown Some suggestions however may be drawn from the fact that it is limited to the periods when the uterus exhibits rhythmic activity *i e* when it carries on its functions of transport of sperma from vagina to the tubes or expulsion of the uterine content during partus Activation of the sympathetic nervous system during sexual excitement may play a role in the former and the association with labour pain in the latter

(ii) *The excitatory oxytocin response* The hypothalamic regions capable of influencing the oxytocin secretion have been studied by Cross (1958) using the milk ejection in lactating animals as an indicator In mapping of hypothalamic regions giving the oxytocin response alteration in uterine motility in the estrogenized rabbit seems to be less suitable because the sympathetic responses tend to dominate Cross (1958) found a considerable overlapping between the hypothalamic regions from which discharge of oxytocin was evoked and those from which sympathetic responses were elicited He also found that sympathetic activation inhibited the uterine and mammary responses to oxytocin a phenomenon which he correlated with the emotional inhibition of milk ejection in the unanesthetized animal (Cross 1955)

(iii) In the present study an *inhibition* of the rhythmic uterine motility has to our knowledge been demonstrated for the first time by mean of central nervous stimulation The regions found to evoke such effects appear to correspond to those previously known

to exert inhibition on other autonomic activities i e the midline structures of medulla oblongata (Alexander 1946 Kuru *et al* 1959) lobus medius medianus and lobus ansoparamedianus of the cerebellum (Moruzzi 1940 Zanchetti and Zaccolini 1954 Sawyer Hillard and Ban 1961) the hypothalamus (Folkow Johansson and Oberg 1959) and three regions in the cerebral cortex (Kaada 1951 1961) located in the orbital surface the anterior limbic region and the sensorimotor cortex

Alexander (1946) demonstrated that the medulla oblongata exerts a tonic influence on structures receiving sympathetic innervation This tone can be inhibited but it is not known where the inhibition takes place In cats Folkow *et al* (1959) observed depressor responses following stimulation of a restricted area just behind and below the anterior commissure These were unaffected by atropine administration but were blocked by dihydroergotamine indicating that the mechanism underlying the depressor response is a reduction in sympathetic tone They suggested that this area constitutes an hypothalamic relay station for cortico-inhibitory pathways to subordinated sympathetic structures It seems likely that excitation of the same pathways is responsible for the uterine response

With regard to cerebral inhibition on the uterus an indirect evidence for this phenomenon was brought nearly 50 years ago by Barbour and Copenhaver (1916) who found that cooling of the cerebral cortex and the basal ganglia increased the uterine rhythmic activity

The uterine inhibition produced by cerebral stimulation is relatively short lasting suggesting that the extent of nervous dominance on the myogenic rhythmic uterine activity is fairly limited at least in the anesthetized animal In spite of this the cerebral inhibitory influence may well play a role in uterine physiology either by influencing the uterine motility directly or by withdrawal of the sympathetic inhibitory influence on oxytocin secretion

Although the uterus is capable of exerting several of its functions in complete independence from the nervous system the present study has shown that the uterine motility can be influenced from the same regions of the central nervous system which are known to influence other autonomic activities and which may also exert an integrative action on somatic behavioural and emotional activities Therefore the uterus does not possess a unique position compared with other autonomic organs in this respect

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Der Effekt plethysmografischer Füllungsdrucke bis 20 cm H_2O auf die lokale Extremitätendurchblutung

Von

Klaus Graf und Sune Rosell

Eingegangen am 19. März 1964

Abstract

Graf, K. and S. Rosell. *Der Effekt plethysmografischer Füllungsdrucke bis 20 cm H₂O auf die lokale Extremitätendurchblutung.* Acta physiol. scand. 1964. 62. 323—335. — The local circulatory effects of the pressure in flexible air filled segmental plethysmographs applied to the human forearm and the hind leg of the cat were investigated. The rate of arterial inflow was measured both by means of a plethysmograph and by either thermal conductivity recorders or a drop recorder. The forearm blood volume capacity was measured. Pressures in the plethysmograph less than 5 cm H_2O did not affect the circulation. In the forearm pressures in the plethysmograph of 10 and 20 cm H_2O caused partial venous occlusion and thereby reduced the local vascular capacity by about 10—15 and 30—35 per cent respectively. Raising the plethysmograph pressure from 5 to 10 cm H_2O did not influence the arterial inflow rate or produced a slight increase. However, after deep forearm nerve blockade a decrease of arterial inflow rate was produced. At pressures in the plethysmograph higher than 10—15 cm H_2O a reduction of the arterial inflow in the forearm usually occurred and was probably more pronounced in the vessels of the skin than those of the deeper tissues. The local effects on arterial inflow of plethysmograph filling pressures higher than 5 cm H_2O may therefore consist of a simultaneous tendency to mechanical obstruction of outflow (passive component) and a counteracting vasodilatation of the resistance vessels (reactive component) which compensates or overcompensates within a certain range for the increasing filling pressure.

Luft- oder wassergefüllte Plethysmografen, die am häufigsten zur quantitativen Messung der menschlichen Extremitätendurchblutung verwandt werden, arbeiten gewöhnlich mit Füllungsdrucken bis zu ca. 20 cm H_2O (15 mm Hg) und üben folglich einen gleichgrossen oder etwas geringeren Belastungsdruck auf die Gefässe des plethysmografischen Messbereiches aus. Der transmurale Druck dieser Gefässe wird dadurch vermindert, was bei passiv-elastischem Gefässverhalten zu Gefässverengerung und Durchblutungsabnahme im plethysmografischen Messbereich führen müsste.

Hingegen fand Wallace (1958) mit wassergefüllten Plethysmografen am Unterarm, dass hydrostatische Druckbelastung bis zu 16 cm H_2O noch zu gar keiner und weitere Druckbelastung bis zu 26 cm H_2O nur zu einer geringen Durchblutungsverminderung von 0,026 ml/100 ml min per 1 cm Druckerhöhung führte. Wallace (1958) nahm daher an, "that there may be some compensation in the circulation for the probable slight reduction in perfusion pressure". Ähnliche Ergebnisse hatten Halperin *et al.* (1948) und Hyman *et al.* (1963) gefunden.

Tonisierte Widerstandsgefässe können, wie gut bekannt ist, auf Verminderung des transmuralen Druckes mit Tonusabnahme reagieren, wodurch ihr Kaliber gleich bleiben oder bei überschiessender Tonusabnahme *sogar auch zunehmen kann* (cf. Folkow 1962). So sahen Langendorf *et al.* (1955) an den Schwimmhautgefässen des Frosches bei Erhöhung des Aussendruckes auf 10 cm H_2O eine Kapillarerweiterung um 24 % und damit verbunden *einen lokalen Durchblutungsanstieg*. Gleichartige Durchblutungsreaktionen wurden an den Extremitäten von Mensch und Katze gefunden (Folkow 1949, Golenhofen und Hildebrandt 1957 a und b, weitere Literatur in Diskussion.) Daher lag die *Annahme nahe* — und wurde in diesen Untersuchungen bestätigt — dass auch die üblichen plethysmografischen Füllungsdrucke bis 20 cm H_2O zu ähnlichen Durchblutungsreaktionen führen konnten. Die genauere Kenntnis solcher *Beeinflussungen von Grösse und womöglich auch Reagibilität der lokalen Durchblutung* durch das plethysmografische Messverfahren selbst ist aber für die Bewertung okklusionsplethysmografischer Durchblutungsmessungen von erheblicher Bedeutung.

Methodik

Messungen am Unterarm

Die Versuche erfolgten nach einer initialen Ruheperiode von mindestens 30 min an 28 ruhig liegenden gesunden Personen (Studenten). Störungen durch die Umgebung oder unbequeme Lagerung wurden sorgfältig vermieden. Zimmertemperatur 23 °C.

Durchblutungsmessungen mit venöser Okklusionsplethysmografie erfolgten am proximalen Teil des rechten Unterarmes mit 5 cm langen luftgefüllten flexiblen Segmentplethysmografen nach Dohn (Einzelheiten bei Graf und Westersten 1959). Der Unterarm war in einer Höhe von ca. 5 cm über dem Niveau des Manubrium sterni gelagert. Die Staumanschette am Oberarm sass unmittelbar oberhalb des Ellenbogens, 1—2 cm distal vom Plethysmografen war eine Manschette zur Blockade des venösen Rückflusses in das Messgebiet während der Durchblutungsmessungen angebracht. Die beiden Okklusionsmanschetten wurden entweder synchron auf *gleichen Druck von meist 70 mm Hg gefüllt* oder die distale Manschette wurde bereits 1 min vor Beginn der plethysmografischen Durchblutungsmessungen auf 70—100 mm Hg aufgeblasen (Graf 1964 a).

Bei 21 Personen erfolgten die Messungen mit zwei der je 5 cm langen Segmentplethysmografen. Diese waren am proximalen Teil des Unterarmes unmittelbar hintereinander angebracht und parallel gekoppelt, was die Länge des plethysmografischen Messbereiches auf 10 cm verdoppelte. Wurde in diesen Versuchen nur mit einem Plethysmografen gemessen (Tab. II), dann mit dem distalen, 1—2 cm distal von diesem befand sich die distale Okklusionsmanschette.

Auf jeder Füllungsdruckstufe wurden 1—2 min nach Änderung des Füllungsdruckes plethysmografisch 5—8 Durchblutungswerte registriert.

Durchblutungsmessungen mit Wärmetransport ahlmessern (WTM) nach Hensel erfolgten — bei konstantem distalen Okklusionsdruck von 70 bis 100 mm Hg — bei 10 Personen. Die WTM wurden im plethysmografischen Messbereich am Unterarm plaziert (bei 2 hintereinander angebracht *n* Plethysmografen unter dem proximalen). Der Muskel-WTM (Sonde) wurde vom proximalen Rand des Plethysmografen aus schräg in die Brachioradialis-Extensorenmuskulatur eingeführt, der Haut-WTM (Platte) unter die innere Lamelle des Plethysmografen geschoben. Die Plazierung war identisch mit der schematischen Darstellung in Fig. 1 bei Graf (1964 a). Die WTM-Messungen erfolgten nicht gleichzeitig mit den plethysmografischen Durchblutungs-

messungen Naheres zur Registrierung und Auswertung von Warmetransportzahlmessungen bei Golenhofen et al (1963)

Der Haut WTM verzeichnete bei steigenden Fullungsdrucken stets erhebliche Zunahmen der Warmeleitzahlwerte (bei Fullungsdrucken von 4 cm H_2O + 14(5—73) bei Fullungsdrucken von 10 cm H_2O + 32(5—50)% bei Fullungsdrucken von 15 cm H_2O + 40 (8—68) Mittelwerte und Bereiche von Messungen bei 8 Personen in der bei Fullungsdruck Null gemessenen mittleren Ausgangswerte) Diese Werte konnen hingegen nicht allein auf die Hautdurchblutung bezogen werden weil der unter dem Segmentplethysmografen liegende Haut WTM mit steigendem Fullungsdruck immer starker der Haut angepresst wurde was den Abstand zwischen Messort und Gefassen verringerte und die Warmeubergangsbedingungen verbessern konnte (Graf und Rosell 1958)

Nervenblockade Bei 7 Personen wurde die Unterarmdurchblutung (mit 2 zusammengekoppelten Segmentplethysmografen) gemessen vor sowie 30 min nach temporarer Blockade der Nn radialis ulnaris und medianus (deep forearm nerves) am Oberarm Die Blockade erfolgte durch intra und perineurale Injektion von Carbocain® 1 (Bofors) nach der Technik von Barcroft und Swan (1953) Das Kriterium ausreichender Nervenblockade war eine nach 15—30 min praktisch komplette und dann etwa 60 min anhaltende motorische Unterarmlahmung

Der *Fullungsdruck* der Segmentplethysmografen wurde zwischen 0 und 20 cm H_2O variiert und kontinuierlich mit einem empfindlichen Anaeroidbarometer (Diameter 15 cm Skalenunterteilung 2 mm H_2O) kontrolliert Mit den Durchblutungsmessungen wurde stets erst 1—2 min nach Anderungen des Fullungsdruckes begonnen

Registrierung Plethysmogramm und Druck in der Okklusionsmanschette am Oberarm wurden mit einem 4 Kanal Direktschreiber (Mingograf plus Elektromanometer 42 lineare Schreibbreite fur jeden Kanal 6—8 cm Elema Stockholm Solna) registriert Die Warmetransportzahlregistrierungen erfolgten auf einem 2 Kanal Direktschreiber (Fluvograph Hartmann & Braun Frankfurt) Registrierbeispiele in Fig 1 und 6

Messungen an der Hinterextremitat der Katze

Bei 3 Katzen wurde die A femoralis dx im unteren Drittel des Oberschenkels ligiert und die arterielle Durchblutung zum Unterschenkel durch einen fotoelektrischen Tropfenzahler geleitet der einen Ordinatenschreiber steuerte Der arterielle Einstrom in den rechten Unterschenkel wurde in einem 5 cm langen luftgefüllten Dohrn chen Segmentplethysmografen gemessen der die Wade in ihrer gesamten Länge umschloss (cf Graf und Rosell 1964 Fig 1) Ein cm distal vom Plethysmografen war die Durchblutung durch eine Ligatur unterbunden Das Volumen des plethysmografischen Messbereiches betrug im Durchschnitt 20 ml der Bereich fur die Messung des arteriellen Einstromes mit dem Tropfenzahler war um ca 35(25—40) grosser Die Okklusionen ur plethysmografischen Durchblutungsmessung erfolgten in Oberschenkelmitte 3—5 cm proximal vom Abgang der zum Tropfenzahler fuhrenden Femoraliskanule Der Druck in der A carotis lag zwischen 160 und 100 mm Hg Plethysmogramm und Okklusionsdruck wurden mit derselben Apparatur wie am Unterarm registriert (Registrierbeispiele in Fig 6 B) Alle weiteren methodischen Daten dieser Versuche sind identisch mit den bei Graf und Rosell (1964) beschriebenen

Der plethysmografische Fullungsdruck wurde zwischen 0 und 20 cm H_2O variiert Auf jeder Druckstufe wurden 1— min nach der Anderung des Fullungsdruckes plethysmografisch 3—5 Durchblutungswerte registriert

Ergebnisse

Effekte auf die Durchblutung (Arterieller Einstrom)

Plötzliche Anderungen des plethysmografischen Fullungsdruckes fuhrten ab ca 6 cm H_2O in der Muskulatur des plethysmografischen Messbereiches zu sofort einsetzenden oft zweiphasigen Durchblutungsanderungen (Fig 1) Bei Erhohung des Fullungsdruckes erfolgte in den ersten 5—20 sec ein geringer Durchblutungsabfall wonach die Durchblutung wieder bis zum Ausgangswert oder auch uber diesen hinaus anstieg (Fig 1) Umgekehrte Durchblutungsanderungen erfolgten bei plotzlicher Verminde

rung des Füllungsdruckes (Fig 1) Stationäre Endwerte (abgesehen von den üblichen Spontanschwankungen) wurden frühestens 20—50 sec nach Füllungsdruckänderungen erreicht und blieben dann während Registrierungen bis zu 15 min Dauer gewöhnlich unverändert bestehen

Die im Unterarm (plethysmografische Messung) in verschiedenen Versuchsreihen erhaltenen stationären Durchblutungswerte waren recht gleichartig (Tab I und II Fig 2 4 und 7) Die Durchblutung stieg ab Füllungsdrucken von 4—6 cm H_2O im Durchschnitt leicht an erreichte bei Füllungsdrucken von 8 (6—10) cm H_2O ein Maximum und fiel bei weiterer Druckerhöhung wieder allmählich ab Die Ausgangswerte wurden meist erst bei Füllungsdrucken von 10—15 cm H_2O unterschritten

Plethysmografische Füllungsdrucke bis 4 cm H_2O hatten noch keinen sicheren Effekt auf die Unterarmdurchblutung (Tab I und II Fig 2) Die bei Füllungsdrucken bis 4 cm H_2O registrierten geringen Schwankungen der lokalen Muskeldurchblutung von im Durchschnitt ± 5 % (Fig 2) können wahrscheinlich noch den üblichen spontanen Muskeldurchblutungsschwankungen zugerechnet werden

Tab I *Durchblutung in Unterarm und Unterarmmuskulatur bei steigendem plethysmografischen Füllungsdruck* Werte aus denselben Versuchen wie in Fig 2

a *Mittelwerte (M) Standardabweichungen (s) und Mittelwerte der individuellen Prozentwert (M in [illegible]) von 12 Messungen an 9 Personen 100 [illegible] = Unterarmdurchblutung bei 2 cm H_2O Muskeldurchblutung bei 0 cm H_2O*

		Plethysmografischer Füllungsdruck cm H_2O							
		0	2	4	6	8	10	15	20
Unterarmdurchblutung	*M*	—	*3 1*	*3 1*	*3 [illegible]*	*3 [illegible]*	*3 1*	*3 0*	*[illegible]*
ml 100 ml min	s	—	0 9	0 8	0 9	0 9	0 8	0 8	0 8
	M in	—	*100*	*100*	*101*	*103*	*100*	*[illegible]5*	*88*
Muskeldurchblutung im	*M*	*100*	*100*	*[illegible]*	*10[illegible]*	*104*	*101*	*[illegible]*	*[illegible]3*
Unterarm de mittleren Ausgangswerte	s	—	7	9	1[illegible]	18	[illegible]	11	11

b) Werte der Muskeldurchblutung di[illegible] bei 3 dieser 9 Personen mit jeweils 2 verschiedenen Lagen der Muskelsonde (I und II) gemessen wurden In Fig 2 sind die im oberen Abschnitt angegebenen Werte mit ● die in der Mitte mit [illegible] und die unten mit ▲ bezeichnet

		0	2	4	6	8	10	15	20
Muskeldurchblutung im	I	100	100	100	10[illegible]	113	114	1[illegible]0	113
Unterarm % des mittleren Ausgangswertes	II	100	100	10[illegible]	[illegible]8	104	90	[illegible]	84
	I	100	96	10[illegible]	108	110	11[illegible]	10	100
	II	100	98	9[illegible]	[illegible]8	[illegible]	94	[illegible]	—
	I	100	—	[illegible]8	10[illegible]	10[illegible]	98	10[illegible]	88
	II	100	93	100	94	90	[illegible]	9	81

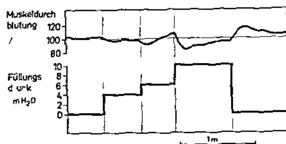


Fig 1 Verhalten der Muskel durchblutung (Warmeleitmessung) im plethysmografischen Messbereich am Unterarm bei Anderungen des plethysmografischen Fullungsdruckes Verwandt wurden 2 hintereinander angebrachte Segmentplethysmografen Kalibration in / des mittleren Ausgangswertes

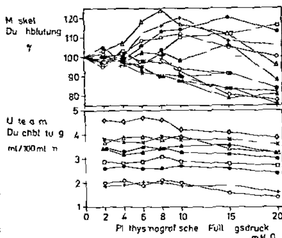


Fig 2 Durchblutung im Unterarm (2 Segmentplethysmografen) und in der Muskulatur des plethysmografischen Messbereiches (Warmeleitmessung Kalibration in des mittleren Ausgangswertes bei Fullungsdruck Null) bei steigendem plethysmografischen Fullungsdruck Durchschnitt der bei jeder Versuchsperson 1—4 min nach Anderungen des Fullungsdruckes registrierten Einzelwerte Plethysmografische und Warmeleitmessung n erfolgten nicht gleichzeitig 12 Messungen an 9 Personen bei 3 Personen (bezeichnet durch die Symbole O ● und ▲ Werte in Tab 1 b) erfolgten jeweils 2 Messreihen mit unterschiedlichen Lagen der Muskelsond

Im Gegensatz zu den im gesamten Unterarmsegment plethysmografisch registrierten Durchblutungsreaktionen waren die lokal in der Unterarmmuskulatur gemessenen Durchblutungsanderungen nicht immer einheitlich (Tab I Fig 2) Bei einigen Sondenlagen wurde ein ahnliches Durchblutungsverhalten wie bei der Gesamtdurchblutung gefunden bei anderen Sondenlagen dagegen wurde die lokale Muskeldurchblutung durch steigende Fullungsdrucke nur zunehmend vermindert Dieser Unterschied war besonders deutlich erkennbar bei Versuchen an 3 Personen bei denen die Muskeldurchblutungsreaktionen am selben Arm nacheinander mit jeweils 2 verschiedenen Lagen der Warmeleitsonde registriert wurden (Tab I b Fig 2) dabei wurde sorgfaltig gepruft dass nur einwandfrei zur Durchblutungsregistrierung geeignete Sonden la en verwandt wurden cf Graf und Rosell 1964) In allen 3 Fallen wurde bei Fullungsdrucken bis zu 15 cm H_2O mit der einen Sondenlage ein Durchblutungsanstieg registriert wahrend mit der anderen Sondenlage ein erheblich geringerer Durchblutungsanstieg (Versuch ● oder aber nur starker zunehmende Durchblutungsverminderungen (Versuche O und ▲ in Tab I b und Fig 2 gefunden wurden Diese mit jeweils 2 Sondenlagen erhaltenen Durchblutungswerte differierten bei Fullungsdrucken bis 20 cm H O um 18—29 (Tab I b)

TAB II Unterarmdurchblutung bei steigendem plethysmografischen Füllungsdruck. Auf jeder Druckstufe erfolgten 1—2 min nach Änderung des Füllungsdruckes 5—8 Einzelregistrierungen erst mit dem distalen, anschliessend mit beiden hintereinander angebrachten Plethysmografen gleichzeitig. Messungen an 8 Personen. Mittelwerte (M), Standardabweichungen (s) und Mittelwerte der individuellen Prozentwerte (M in %) 100 = Werte bei 2 cm H_2O

Unterarmdurchblutung ml/100 ml min		Plethysmografischer Füllungsdruck cm H_2O						
		2	4	6	8	10	15	20
1 Segmentplethysmograf	*M*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	s	1 0	0 9	1 0	1 0	1 0	0 9	0 9
	M in %	*100*	[illegible]	*101*	*101*	*100*	[illegible]	[illegible]
2 Segmentplethysmografen	*M*	[illegible]	[illegible]	*2 8*	*2 8*	[illegible]	[illegible]	[illegible]
	s	*1 0*	*1 0*	*1 0*	*1 1*	*1 0*	*0 9*	*0 8*
	M in %	*100*	[illegible]	[illegible]	*102*	*102*	[illegible]	[illegible]

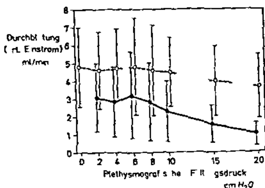
Die Grösse der Unterarmdurchblutungsänderungen war vermutlich von der Länge des plethysmografischen Messbereiches abhängig. Der Durchblutungsanstieg bei Füllungsdrucken von 6—10 cm H_2O und der bei höheren Füllungsdrucken einsetzende Durchblutungsabfall waren angedeutet stärker, wenn mit zwei unmittelbar hintereinander angebrachten und parallel zusammengekoppelten 5 cm langen Segmentplethysmografen gemessen wurde (Tab. II). Die im etwa doppelt so grossen Messbereich *erhaltenen Werte der Unterarmdurchblutung (in ml/100 ml min) waren im* Durchschnitt annähernd gleich gross wie die mit nur einem 5 cm langen Segmentplethysmografen erhaltenen Werte (Tab. II).

Ähnliche Durchblutungsänderungen wie am Unterarm wurden bei Erhöhung des plethysmografischen Füllungsdruckes bis 20 cm H_2O auch am Katzenbein gefunden (Fig. 3). Die plethysmografisch am Unterschenkel der Katze gemessenen Durchblutungsänderungen waren angedeutet grösser als die mit dem Tropfenzähler registrierten, was damit erklärt werden kann, dass der ca. 3 [illegible] grösserer Bereich für die arterielle Einstrommessung mit dem Tropfenzähler nicht vollständig dem plethysmografischen Füllungsdruck ausgesetzt war.

Nervenblockade (Blockade der sympathischen Vasokonstriktornerven) am Unterarm führte zu einer angedeuteten Veränderung des Durchblutungsverhaltens bei steigendem plethysmografischen Füllungsdruck (Fig. 4). In Versuchen an 7 Personen zeigte die Unterarmdurchblutung bei Füllungsdruckerhöhungen bis zu 8—10 cm H_2O vor der *Nervenblockade* die oben beschriebene Tendenz zu geringem Anstieg, während sie an denselben Armen nach Nervenblockade mit steigendem Füllungsdruck in eher mehr linearer Weise abfiel (Fig. 4).

EFFEKTE AUF DIE GEFÄSSKAPAZITÄT

Als relatives Mass für die *Gefässkapazität* diente der Anstieg des Unterarmvolumens während 1 minutiger Okklusion am Oberarm mit Manschettendrucken bis zu 50 mm Hg. Plethysmografische Füllungsdrucke von 2 und 4 cm H_2O hatten noch keinen er



F g 3 Durchblutung (arterieller Einstrom) in der Hinterextremitat der Katze bei steigendem plethysmografischen Fullungsdruck am Unterschenkel O = Tropfenmessung ● = plethysmografische Messung im ca 3ↄ kleineren Messbe re ch am Unterschenkel Vornahme der Messungen 1—2 *min nach* Anderungen des Fullungsdruckes Mittelwerte und e nfache *Standard* abweichungen aus 9 Messreihen an 3 Katzen auf jeder Druckstufe wurden 3—5 plethysmografische Einzelwerte registriert

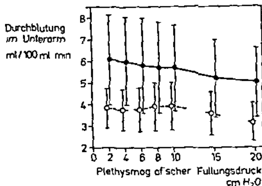
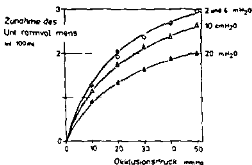


Fig 4 *Unterarmdurchblutung* (Messungen mit 2 hintereinander ang brachten *Segmentplethysmografen*) bei steigendem plethysmografischen Fullungsdruck D e Messungen *erfolgten jeweils am selben* Arm vor (O) und 30—60 m n nach (●) Nervenblockade im Unterarm (deep forearm nerves) Untersu chungen an 7 Personen Mittelwerte und e nfache Standardab eichungen



F g 5 Zunahme des Unterarm volumens be versch edenen Okklu *sionsdrucken am Oberarm Be* hoheren Fullungsdru ken im Plethysmografen (O = ʾ cm H O ● = 4 cm H O △ = 10 cm H O ▲ = 0 cm H O) wu den die Volumen nst ege geringer M ttel erte aus 12 Messungen n 8 Personen D e Mes *ungen erf lgt n m t e nem 5 m* b eiten Segm ntpl tl vsmografen Ablesung der Werte jeweils 1 m n na h E höhung des Okklus ons Iru kes

kennbaren Effekt auf die Gefasskapazitat im Unterarmsegm nt oder ermind rten d ese *nur minimal (Fig 5) Fullungsdrucke* von 10 cm H_2O *verminderten die* Gefasskapazitat um ca 10—15 Fullungsdrucke von ⁿ0 cm H O bereits um a 30—35 gegenuber den bei Fullungsdrucken von 2 und 4 cm H_2O rhaltenen W rten Fig 5

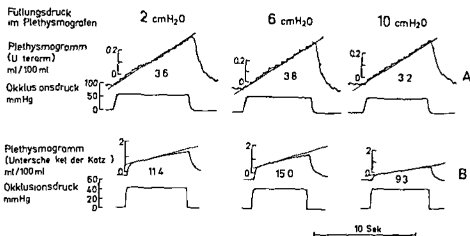


Fig 6 Okklusionsplethysmografische Einflusskurven bei plethysmografischen Füllungsdrucken von 2 6 und 10 cm H O Registrierungen aus je einem Versuch (A) am Unterarm (B) am Unterschenkel der Katze (Messbereichgrosse 14 ml) Unterschiedlicher Verstarkungsgrad der *Volumenregistrierung bei verschiedenen Füllungsdrucken* Unter den Einflusskurven Angabe der Durchblutungswerte in ml/100 ml min

Die Verminderung der Gefässkapazität um 10—15 % durch Füllungsdrucke bis 10 cm H_2O verringerte noch nicht die Möglichkeit zur Registrierung linearer okklusionsplethysmografischer Einflusskurven weder bei normal grosser Durchblutung (Unterarm) noch bei etwas erhöhter Durchblutung (Unterschenkel der Katze) (Fig 6)

Plethysmografische Füllungsdrucke die zu distalem Volumenanstieg fuhren mussen die Venen im plethysmografischen Messbereich verengern und dadurch den lokalen venosen Stromungswiderstand erhohen Am Unterarm wurde der geringste plethysmografische *Füllungsdruck der schon einen distalen Volumenanstieg bewirkte mit Hilfe* von 2 unmittelbar hintereinander angebrachten 5 cm langen Segmentplethysmografen bestimmt Mit dem distalen Plethysmografen (Füllungsdruck 2 cm H_2O) wurden die Änderungen des Unterarmvolumens registriert wahrend im proximalen Plethysmografen der Füllungsdruck in Stufen von 0 5 cm H_2O erhoht wurde Ein distaler Volumenanstieg erfolgte erst ab Füllungs (Okklusions)Drucken von 6 0 (5 0—9 5) cm H O (Mittelwert und Bereich aus Messungen an 18 Personen) Plethysmografische Füllungsdrucke bis 5 cm H O fuhren also am Unterarm noch nicht zu einer sicheren Erhohung des venosen Stromungswiderstandes verengern folglich noch nicht die venosen Gefasse und vermindern somit auch noch nicht die Gefasskapazität was mit den in Fig 5 gezeigten Befunden ubereinstimmt

Diskussion

Zur Natur der Durchblutungsreaktionen

1 Änderungen des (arteriellen) Gefässwiderstandes

Arterieller Druck und Blutviskosität wurden unter den Bedingungen dieser Versuche nicht beeinflusst weshalb die Durchblutungskonstanz oder auch Durchblutungszunahme die trotz des Anstieges des plethysmografischen Füllungsdruckes beobachtet

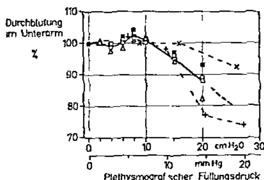


Fig 7 Gesamtdurchblutung und Muskeldurchblutung im Unterarm (in % des Ausgangswertes bei 0 resp 2 cm H_2O) in Abhängigkeit vom plethysmografischen Füllungsdruck Eingezeichnet sind die Werte aus folgenden Untersuchungen O (Tab II plethysmografische Messung Mittelwerte beider Messreihen) □ (Tab I a plethysmografische Messung) ■ (Tab I a Warmeleitmessung im Muskel) △ (Fig 4 unbeeinflusster Arm) x (nach Wallace 1958) + (Durchschnittswerte nach Hyman *et al* 1963 Fig 4)

wurde durch eine Widerstandsabnahme (Tonusabnahme der Widerstandsgefässe) erklart werden muss Diese Widerstandsabnahme war so gross dass sie auch den durch die gleichzeitige druckpassive Venenverengerung erhohten venosen Gefasswiderstand kompensierte oder bei Durchblutungsanstieg sogar uberkompensierte

An ausreichend tonisierten Widerstandsgefässen konnen wie gut bekannt ist solche Widerstandsanderungen durch Änderungen des transmuralen Druckes (TMD) ausgelöst werden wobei Abnahme des TMD mit einer lokalen („myogenen") Tonusverminderung Zunahme des TMD dagegen mit einer Tonuserhohung beantwortet werden kann (cf Folkow 1949) Auslosender Faktor fur die hier beobachteten Widerstandsabnahmen konnte also der plethysmografische Fullungsdruck oder anders ausgedruckt die durch ihn bewirkte Verminderung des TMD der Widerstandsgefässe gewesen sein Am Unterarm und Katzenbein erreichten diese Widerstandsabnahmen bei Fullungsdrucken von ca 8—10 cm H_2O ein Maximum weshalb bis dorthin die Durchblutung gleichbleiben oder bei uberschiessender Tonusabnahme sogar auch zunehmen konnte (Fig 7) Bei noch hoheren Fullungsdrucken war eine weitere derartige Tonusverminderung nicht mehr moglich die arteriellen Widerstandsgefässe wurden nun zunehmend druckpassiv verengt und die Durchblutung nahm ab ein Reaktionsverhalten welches auch mit den von anderen Autoren gefundenen Ergebnissen grundsatzlich ubereinstimmte (Halperin *et al* 1948 Wallace 1958 Hyman *et al* 1963 und 1964 siehe auch Fig 7) Grossere Arterien werden im Unterarm durch Fullungsdrucke bis 20 cm H_2O noch nicht messbar verengt (Graf und Rosell 1964)

Widerstandsanderungen dieser Art erfolgen in der Hauptsache an den präkapillaren Widerstandsgefässen (Folkow 1962) womoglich auch an Kapillaren und Venolen (Langendorf *et al* 1955) Sie sind ein verbreitetes Phanomen das — mit Widerstandsabnahmen bei Verminderung des TMD wie Widerstandszunahmen bei Erhohung des TMD — vor allem an den Gefässen von Skeletmuskulatur und Haut (Folkow 1949 Golenhofen und Hildebrandt 1957 Hildebrandt 1960) aber auch an denen von Lunge Niere Intestinum und Schwimmhaut des Frosches (Folkow 1949 Wezler und Sinn 1953 Langendorf *et al* 1955 und 1956 Henne und Thurau 1964) beobachtet wurde Gleichartige Reaktionen (als Widerstandszunahmen bei Erhohungen des TMD) wurden auch an Unterarm Wade Hand Finger und Fuss gefunden (Greenfield und Patterson 1954 Coles und Greenfield 1956 Blair *et al* 1959 Golenhofen 1962)

2 *Der zeitliche Verlauf der Durchblutungsanderungen*

Auch der zeitliche Verlauf dieser Durchblutungsanderungen deutete auf durch Änderungen des TMD ausgeloste Widerstandsanderungen hin die Durchblutungsreaktionen

setzten praktisch sofort bei Änderungen des TMD ein, verliefen initial aber in anderer Richtung ein Durchblutungsverhalten, das als Reaktion auf Änderungen des TMD gut bekannt ist (Folkow 1949 Greenfield und Patterson 1954 Langendorf *et al* 1955 Golenhofen und Hildebrandt 1957 Blair *et al* 1959) Bei konstantem TMD konnen die (nach fruhestens 20—60 sec) stationaren Durchblutungswerte wahrscheinlich uber langere Zeit beibehalten werden in diesen Untersuchungen hielten sie bis zu 15 min (*langer wurde nicht gemessen*) bei *Langendorf et al* (1955 und 1956) wahrend mehrerer Stunden im wesentlichen unverandert an Sogar die chronische Erhohung des arteriellen Stromungswiderstandes im Arm bei Coarctatio aortae wird als eine durch den erhohten arteriellen TMD ausgeloste *Tonuszunahme der arteriellen Widerstands*gefasse aufgefasst (cf Shepherd 1963)

3 Gefasstonus

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1 Effekt auf die Haut und Muskeldurchblutung

Der effektive aussere Belastungsdruck nimmt radiar ab (bis in Mitte des Unterarmsegmentes um maximal 20—40 % cf Graf und Rosell 1964) weshalb plethysmografische Fullungsdrucke bevorzugt die Hautdurchblutung beeinflussen Die hier angestellten Versuche konnten nichts uber das Verhalten der Hautdurchblutung bei steigendem plethysmografischen *Fullungsdruck* aussagen (weil der Haut Warmeleitmesser immer starker der Haut angepresst wurde siehe unter Methodik) Andere Untersuchungen (siehe oben) haben aber ergeben dass auch die Hautgefässe auf Änderungen des TMD

in gleicher Weise reagieren können wie die Gefässe in Muskulatur und anderen Organen. Am Unterarm wird aber die Hautdurchblutung ab Füllungsdrücken von 5—10 mm Hg (7—14 cm H_2O) bereits sicher reduziert (Hyman *et al.* 1963), wobei unter Umständen gleichzeitig eine geringe Zunahme der Durchblutung tieferer Gefässe (Muskel, Knochen) erfolgen kann. Mit Füllungsdrücken von mehr als 7—14 cm H_2O werden plethysmografisch also vor allem Grösse und Verhalten der Muskeldurchblutung erfasst (siehe auch Golenhofen und Mitsany 1962), wobei freilich der Füllungsdruck besonders im Hinblick auf die tieferen Gefässe nicht die Höhe des critical closing pressure (am Unterarm 33 (10—68) mm Hg, Ashton 1963) erreichen darf.

2. *Effekt auf die Gefässkapazität im Unterarm*

Ein distaler Volumenanstieg — und somit eine Venenverengerung und Verminderung des Füllungsvolumens der Gefässe im plethysmografischen Messbereich — erfolgte am Unterarm erst ab Füllungsdrücken von 5 cm H_2O, Werten, die sicher denen des lokalen Venendruckes nahekamen. Für die Registrierung einwandfreier okklusionsplethysmografischer Einflusskurven ist aber ein ausreichend grosses Füllungsvolumen der kapazitiven Gefässe erforderlich, das am Unterarm mindestens 0,5—1,0 Vol % betragen soll (Graf 1964 b). Das Füllungsvolumen der kapazitiven Gefässe an Unterarm und Unterschenkel der Katze ist dazu bis zu Füllungsdrücken von 10 cm H_2O offenbar noch voll ausreichend, auch bei erhöhter Druchblutung (Fig. 6). Auch bei Füllungsdrücken von 10—20 cm H_2O können dort bei normal grosser Durchblutung noch gut auswertbare Einflusskurven registriert werden. Indes wird das Füllungsvolumen bei einem Füllungsdruck von 20 cm H_2O schon soweit reduziert (bei 1 minutiger venöser Okklusion und normal grosser Durchblutung um ca. 30—35 %, Fig. 5), dass hier bei erhöhter Durchblutung unter Umständen mit verkürzten, nicht mehr hinreichend auswertbaren Einflusskurven gerechnet werden muss.

3. *Lange des Segmentplethysmografen*

Der Belastungsdruck nimmt ausser in radiärer Richtung auch vom Plethysmografenrand einwärts zur Segmentachse hin ab (cf. Graf und Rosell 1964). Der Füllungsdruck von 8 cm langen (Hyman und Winsor 1960), besonders aber derjenige der meist 13—15 cm langen wassergefüllten Plethysmografen (cf. Greenfield *et al.* 1963) muss folglich stärker auf die Gefässe des Messbereiches einwirken als der von nur 5 cm langen Plethysmografen, wie sie in diesen Untersuchungen sowie von Barbey und Barbey (1963) verwandt wurden. Dies mag erklären, warum mit 2 zusammengekoppelten 5 cm lagen Segmentplethysmografen angedeutet grössere Gefässreaktionen als mit nur einem Segmentplethysmografen allein gemessen wurden (Tab. II).

4. *Unterschied zwischen luft- und wassergefüllten Plethysmografen*

Bei wassergefüllten Unterarmplethysmografen ist eine Begrenzung des Füllungsdruckes auf 5 cm H_2O schwierig. Der Mindestfüllungs- (hydrostatische) Druck beträgt gewöhnlich ca. 8,5 cm H_2O (Halperin *et al.* 1948, Wallace 1958); ähnliche Werte können auch für die Modelle anderer Autoren angenommen werden. Da schon dieser Mindestfüllungsdruck den Zustand von Gefässtonus und Durchblutung zu beeinflussen vermag, wird verständlich, weshalb bei Untersuchungen über den Effekt zunehmender hydro-

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From the Department of Pharmacology University of Goteborg Sweden

Reabsorption of Inorganic Sulfate by the Renal Tubules of the Rat

By

FREDRIK BERGLUND[1]

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Abstract

Berglund F *Reabsorption of inorganic sulfate by the renal tubules of the at* Acta physiol scand 1964 6? 336—343 — Reabsorption of inorganic sulfate by the renal tubules was measured in the rat by means of a *constant infusion t chnique Plasma sulfate concentration* v *as varied between* 1 1 and 4 6 μmole/ml Wh n glomerular filtration rate was measured by inul n clearance there seemed to be reabsorption at low sulfate levels but secretion at h gher levels When polyethylene glycol of molecular weight 1 000 or 400 was used instead of inulin the calculated values for glomerular filtration rate were almost twice as high and reabsorption of filtered sulfate exhi bited a Tm (transfer maximum) of 1 02 μmol /ml glomerular filtrate The Tm is of the same order of magnitude as in the dog (1 65 μmole/ml glomerular filtrate) and man (0 97 μmole/ml glomerular filtrate) Carinamide had no effect indicating that tubular secretion of sulf te is ab ent or ins gnificant Neither did phlorizin have any effect contrary to expectations from earlier experiments on the intact dog and on kidney cortex slices of the rat

Reabsorption of inorganic sulfate by the renal tubules has been demonstrated in the dog (Lotspeich 1947) in man (Becker *et al* 1960) and in chicken (Globus Becker and Thompson 1961) A reabsorption Tm (transfer maximum) was demonstrated in all three species Presumably inorganic sulfate is also reabsorbed by the tubules of other mammalian species although no *in vivo* experiments confirm this assumption

Deyrup (1956) measured the *in vitro* uptake of $S^{35}O_4$ in renal cortical slices from vari ous *species* Rat kidney *slices were found to accumulate 3 times as much sulfate as* slices from do kidneys This finding prompted an *in vivo* comparison between th two species

In the present study renal tubular reabsorption of inorganic sulfate was measured in unanesthetized rats Initial experiments with inulin gave peculiar results Polyethyl ene glycol was therefore used in the final experiments for measuring the glomerular filtration rate (GFR) The clearance of polyethylene glycols of average molecular weights from 400 to 4000 are identical to GFR in the dog (Shaffer Critchfield and

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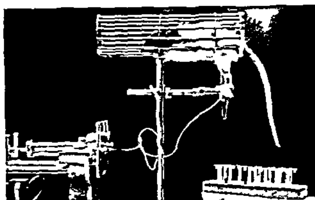


Fig 1 Rat in metal bar cage during clearance experiment Infusion into superficial leg vein

Carpenter 1948) Two glycols with average molecular weights 400 and 1000 respectively (PEG 400 and 1000) were used in the present experiments in the rat

The effects of carinamide L alanine glucose and phlorizin were tested

Methods

Clearance experiments were done on unanesthetized black hooded rats by a technique modified from that earlier described by Berglund (1962) The rat is placed in a metal bar cage (Fig 1) I v infusion is given through a polyethylene catheter (PE 10 inner diameter 0 28 mm outer diameter 0 61 mm) The tip of the catheter is carefully tapered by stretching so as to fit very snugly around a nylon fishing line diameter 0 2 or 0 25 mm Any superficial vein on the hindlegs may be used for the infusions The vein is punctured with a sharp needle and the fishing line is pushed into the vein through the needle After withdrawing the needle the catheter is slid over the nylon line into the vein

In a large number of experiments the GFR was measured by the inulin clearance Inulin was analyzed on trichloroacetic acid filtrates of plasma and urine according to Heyrovsky (1956) and this method was checked with a resorcinol method (Schreiner 1950) Inorganic sulfate was measured by the method of Berglund and Sorbo (1960) Carinamide p-(benzyl sulphonamido) benzoic acid was analyzed in urine according to Brodie Levy and Bernstein (1947)

In the final experiment GFR was measured by the clearance of polyethylene glycol with a molecular weight around 1000 (PEG 1000) or 400 (PEG 400 *PEG 1000* was analyzed by the method of Hyden (1955) but on a simplified sulfate and protein free filtrate

Plasma filtrate Mix 0 1 ml plasma 0 [illegible] ml 6 TCA (trichloroacetic acid) and 0 1 ml 12 BaCl H O After 30 min centrifuge at 4000 rpm for 1 min Filter

Urine filtrate Desired dilutions are made with 4 TCA e 1 volume urine volumes 4 TCA — 1 volume 12 BaCl H O After 30 min centrifuge and filter as with plasma

Turbidimetric analysis To 0 1 ml filtrate add 0 1 ml 6 B Cl H O n 40 TCA Mix Measure turbidity after exactly 10 min (400 m Coleman Junior spectrophotometer with ultramicro cell) Water is used as a blank and standards (0 1 0 [illegible] and 0 3 mg PEG ml are run simultaneously The plasma blank corresponds to a PEG content of 0 0 mg ml The plasma concentrations of PEG in the reported experiments averaged 1 0 mg ml

For analysis of *PEG 400* the gravimetric method of Shaffer Critchfield and Carpenter 1948 was modified to a turbidimetric method This gives straight line standard curves for PEG 400 up to concentrations of 0 07 ml (v/) but is not suitable for analysis of the solid glycols (molecular weight > 1 000)

Plasma filtrate 0 [illegible] ml plasma — 0 6 ml 6 TCA Centrifuge (The filtrate is used also for sulfate analysis) To 0 [illegible] ml supernatant add 1 0 ml 6 BaCl H O n 40 TCA Centrifuge

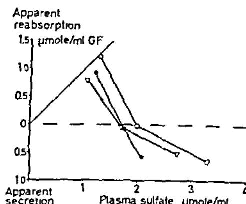


Fig 2 Apparent pattern of reabsorption → secretion obtained in 3 expts when GFR was measured with inulin ●—● exp 206 mannitol diuresis △—△ exp 207 mannitol diuresis ○—○ exp 187 ethanol diuresis Each point represents average of three clearance periods Thin line represents complete reabsorption of filtered sulfate Male rats 305—370 g clearance of inulin 0 74—0 95 ml/min/100 g body weight

Urine filtrate 0 2 ml of suitably diluted urine + 0 2 ml 6 BaCl 2 H O in 40 TCA. Centrifuge

Turbidimetric analysis 0 1 ml filtrate or standard + 0 075 ml 10 silicotungstic acid After approximately 20 min read against H O at 400 mμ Standard solutions of PEG 400 contained 20 TCA and 3 BaCl 2 H O — Plasma blank corresponds to 0 03 μl PEG/ml Concentrations of PEG 400 in the reported experiments averaged 0 4 μl/ml plasma

Statistical analysis was done by standard methods as presented by Snedecor (1956)

Results

Serum level of inorganic sulfate in non fasting rats Blood (0 4 ml) was obtained from 10 male and 10 female rats by cutting the tip of the tail and the sera were analyzed for sulfate The mean value was equal in both sexes and the total average was 0 98 ± 0 07 μmole/ml (mean ± standard deviation)

Reabsorption of inorganic sulfate In the earlier part of the investigation inulin was used for the measurement of GFR In these experiments filtered sulfate was almost completely reabsorbed by the tubules at low plasma levels but at higher levels the reabsorption seemed to reverse to secretion (Fig 2) A definite reabsorption → secretion reversal occurred in 18 out of 55 expts with inulin The pattern was independent of sex or strain of rat type of diuresis and fluid balance Most important however was the finding that carinamide did not inhibit the apparent secretion of sulfate which occurred at high plasma levels

Some technical error was therefore suspected Analyses of inulin and sulfate were checked but the possible errors in these methods could not account for the reabsorption → secretion pattern Such a pattern could however occur if the inulin possibly due to molecular size gave too low values for GFR The clearance of inulin was actually found to be as low as 60 % of the clearance of PEG 1000

When GFR was measured with PEG 1000 instead of inulin more sensible and consistent results were obtained (Table I and Fig 2) At plasma levels between 1 and 5 μmoles/ml the sulfate reabsorption was fairly constant exhibiting a Tm of 1 07 μmole ml glomerular filtrate (ml GF) There was no significant difference between males and females

TABLE I Reabsorption of inorganic sulfate in male and female rats GFR measured with PEG 1000 Each figure in columns 2 and 3 represents average of 3 clearance periods The Tm values represent average reabsorption rate in each experiment

Male rats 245—387 g				Female rats 187—258 g			
Exp	Plasma sulfate (μmole/ml)	Reabsorption (μmole/ml GF)	Tm (μmole/ml GF)	Exp	Plasma sulfate (μmole/ml)	Reabsorption (μmole/ml GF)	Tm (μmole/ml GF)
254	2 02	1 36	1 05	279	1 15	0 82	0 77
	3 19	1 09			2 35	0 77	
	3 66	0 70			3 97	0 71	
257	2 16	1 59	1 71	281	1 20	0 89	0 86
	3 33	1 61			2 34	0 91	
	4 12	1 94			3 83	0 77	
258	1 67	0 99	1 01	283	1 16	0 88	1 19
	2 46	0 97			1 96	1 36	
	3 19	1 07			3 61	1 33	
262	1 33	1 05	1 11	284	1 50	1 22	1 09
	2 74	1 05			2 80	1 16	
	3 75	1 24			4 53	0 88	
264	1 39	1 30	1 30	285	1 28	1 04	1 36
	2 56	1 06			2 80	1 51	
	3 84	1 53			3 97	1 54	
265	1 40	1 22	1 00	286	1 23	0 81	0 85
	2 64	0 67			2 54	0 89	
	3 74	1 12					
267	1 35	1 22	1 30	288	1 40	0 99	0 77
	2 16	1 18			2 31	0 61	
	3 15	1 51			3 59	0 73	
268	1 47	1 28	0 99	292	1 33	1 06	0 91
	2 27	0 89			2 55	0 8	
	3 01	0 81			3 75	0 82	
271	1 20	0 93	0 91				
	2 37	0 87					
	3 60	0 94					

Statistical treatment Mean ± standard deviation

GFR male rats	1 10 ± 0 30 ml min 100 g	Tm male rats	1 15 ± 0 25 μmole ml GF
GFR female rats	0 88 ± 0 08 ml min 100 g	Tm female rats	0 98 ± 0 22 μmole ml GF
GFR male + female	1 00 ± 0 5 ml min 100 g	Tm male + female	1 07 ± 0 24 μmole ml GF
		Tm male / female	P > 0 10

TABLE II Reabsorption of inorganic sulfate in male rats 237—315 g GFR measured with PEG 400

Exp	Plasma sulfate (μmole/ml)	Reabsorption (μmol /ml GF)	Tm (μmole/ml GF)
317	1 23	0 88	1 06
	2 80	0 93	
	4 04	1 36	
318	[illegible]	1 06	1 06
	2 49	1 17	
	3 29	[illegible]	
321	1 23	0 78	[illegible]
	2.28	[illegible]	
	3 12	0 82	
322	[illegible]	0 96	0 94
	[illegible]	[illegible]	
	3 48	1 10	
323	[illegible]	[illegible]	0 72
	2 24	0 52	
	[illegible]	0 66	
[illegible]	1 33	[illegible]	[illegible]
	2 32	[illegible]	
	[illegible]	[illegible]	
[illegible]	[illegible]	1 08	[illegible]
	[illegible]	0 96	
	3 09	0 80	

GFR 1 12 ± 0 23 ml min 100 g

Tm 0 89 ± 0 1[illegible] μmole ml GF

Tm, PEG 400+PEG 1000 series 1 02 ± 0 23 μmole ml GF

Tm, PEG 400 *vs* PEG 1000 series P 0 10

In another series of experiments the GFR was measured with PEG 400 Table II and Fig 3 The reabsorption Tm was slightly lower than in the series with PEG 1000 but the difference was not statistically signif[illegible] The reabsorption Tm from the PEG 400 and PEG 1000 series [illegible] avera[illegible] [illegible]le ml GF

A number of poor experi[illegible] [illegible] the results because of > 100 % difference between sin[illegible] [illegible] one plasma level or between average reabsorption [illegible] [illegible] out of 21 expts in the male rats 7 out of 15 exp[illegible] [illegible] not differ in experiments with unifo[illegible]

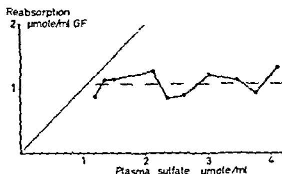


Fig 3 Pattern of reabsorption obtained in 17 rats when GFR was measured with PEG 1000 ●—●—● reabsorption rate calculated from columns 2 and 3 Table I by rearranging in order of rising plasma sulfate and pooling into groups of five — — — —average Tm calculated from Table I

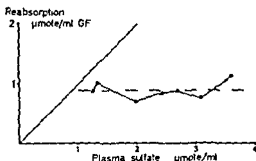


Fig 4 Pattern of reabsorption obtained in 7 rats when GFR was measured with PEG 400 For ●—●—● data from Tabl II were pooled into groups of three — — — —average Tm from Tabl II

Effect of carinamide (Table III) In two experiments carinamide was given i v rats ing the plasma level well above that which fully inhibits secretion of thiosulfate in the dog (20 µg/ml) (Berglund Helander and Howe 1960) No definite effect on sulfate reabsorption was obtained

Effect of L-alanine In one expt 0 66 g L alanine was given i v to a 318 g rat over a 1 hour period producing a copious aminoaciduria Reabsorption of inorganic sulfate dropped from 1 10 to 0 80 µmole/ml GF

Effects of glucose and phlorizin In 4 expts with glucose and 10 with phlorizin (5—100 mg i v) there was either a rise (50) a fall (25) or no change in sulfate reabsorption

Discussion

Magnitude of Tm In the rat kidney filtered sulfate is almost completely reabsorbed by the tubules at low plasma levels *At higher levels a reabsorption Tm is obtained* The normal plasma sulfate level 0 98 µmole ml) is close to the Tm level (1 0? µmole ml GF) Reabsorption Tm therefore may be the principle determinant of the plasma level in the rat This is evidently not the case in other species with plasma levels considerably lower than the Tm level e g in the dog 1 27 µmole/ml plasma (Reed and Denis 19?) cf Tm 1 6? µmole ml GF (Berglund 1960) and in man 0 3? µmole/ml serum (Ollgaard 193?) Tm 0 97 µmole ml GF (Becker *et al* 1960)

TABLE III Effect of carinamide on sulfate reabsorption. Carinamide i.v. 20 mg/kg + 30 mg/kg/hr In each experiment 4 control and 4 drug periods

	Carinamide		Sulfate		
				Reabsorption	
	Plasma	Urine	Plasma	Control	Carinamide
Exp	(μg/ml)	(μg/min)	(μmole/ml)	(μmole/ml GF)	
2?2	26	28	1 8–2 4	1 06 (0 71–1 31)	1 06 (0 81–1 ?0)
273	30	3?	2 0–2 4	1 15 (0 ?7–1 39)	1 27 (1 11–1 34)

Effects of L-alanine glucose and phlorizin in dog and rat Various amino acids depress sulfate reabsorption in the dog (Berglund and Lotspeich 1956) L-alanine which is most effective causes a 75 % inhibition in the dog but only a 27 % inhibition in the rat

In the dog glucose depresses sulfate reabsorption by approximately 25 % whereas phlorizin causes a 120 % enhancement of sulfate reabsorption, presumably by excluding glucose from the tubule cells (Cohen, Berglund and Lotspeich 1956) — In the rat, glucose and phlorizin cause no consistent changes of sulfate reabsorption, although they exert definite effects on the *in vitro* uptake of sulfate by kidney cortex slices At 37 C glucose depresses uptake by 30 whereas phlorizin enhances uptake by 50 (Deyrup 1956) At 0 C glucose has no effect and phlorizin causes a 50 % inhibition (Deyrup and Davies 1961) The dual phlorizin effects *in vitro* may perhaps explain the lack of effect *in vivo* On the other hand, if sulfate reabsorption in the rat is insensitive to endogenous glucose then exclusion of glucose by phlorizin would not necessarily produce any change

It should be recalled that amino acids depress the reabsorption of both creatine and phosphate in the dog (for ref. see Berglund and Lotspeich 1956) Also glucose depresses and phlorizin enhances reabsorption of both phosphate and acetoacetate in the dog (for ref see Cohen *et al* 1956) Thus the effects of amino acids glucose and phlorizin on sulfate reabsorption in the dog are rather nonspecific and the present experiments indicate that they are less pronounced or even absent in the rat

Thiosulfate competes specifically with sulfate transport in the dog (Berglund *et al* 1960) Unfortunately thiosulfate precipitates with PEG 1000 and could therefore not be tested in the rat with the present methods.

Inulin vs polyethylene glycol The present results indicate that inulin clearance may not be a valid measure of GFR in the rat. The discrepancy between inulin and polyethylene glycol is being subjected to further experimental work including comparison between polyethylene glycols and polyfructan molecules of different molecular weights Further discussion on this point does not seem profitable at present.

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The Tissue Distribution and Metabolism in the Rat of Intravenously Injected Labeled Fat Emulsions

By

PER BELFRAGE BO EDGREN and THOMAS OLIVECRONA

Received 24 March 1964

Abstract

Belfrage P B Edgren and T Olivecrona *The tissue distribution and metabolism in the rat of intravenously injected labeled fat emulsions* Acta physiol scand 1964 *62* 344—351 — An artificial fat emulsion containing H palmitic acid labeled triglycerides was injected intravenously into rigorously carbohydrate fed rats One group was given a small dose (0 6 mg lipid) and another group a large dose (136 mg lipid) Radioactivity in blood and tissue lipids was studied at a series of time intervals The label disappeared rapidly from the circulating blood and most of it was taken up by the liver and the spleen The hepatic uptake of radioactivity showed an inverse relationship to dosage while the reverse was true for the splenic uptake In both instances hepatic and splenic uptake was greater than in previous experiments using labeled chylomicrons The label taken up by the liver was first present almost exclusively in the non phospholipid fraction but subsequently label appeared in the liver phospholipids

In a second experiment an artificial soybean emulsion containing tracer amounts of C glycerol H palmitic acid labeled triglycerides was injected into rigorously carbohydrate fed rats In this experiment only the ratios C¹⁴/H in the tissue lipids were studied The data indicated that the fat emulsion triglyceride was taken up without prior hydrolysis into the liver and spleen

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Intravenous injections of artificial fat emulsions have been extensively used to study the removal from the circulation and the tissue distribution of particulate fat (Woerner 1949 Murray and Freeman 1951 Edgren 1960 Carlson and Hallberg 1962) Like chylomicrons fat emulsions rapidly disappear from the blood when injected into experimental animals (Mc Candless and Zilversmit 1958) The emulsions are deposited mainly in the liver and the spleen (Di Luzio 1960 Geyer *et al* 1951) whereas the chylomicrons are removed mainly by the liver and adipose tissue (Bragdon and Gordon 1958 Belfrage Borgström and Olivecrona 1963) It is not clear if the reticulo-endothelial system has an important role in the uptake of fat emulsions (Murray *et al* 1951 Waddell *et al* 1954 Di Luzio 1960)

This paper reports the results of experiments on the tissue distribution and metabolism of radioactively labeled fat emulsions injected intravenously into rats

Materials and methods

Preparation of labeled fat emulsions Two labeled fat emulsions were prepared one containing C glycerol H palmitic acid labeled triglycerides and another tracer amounts of H palmitic acid labeled triglycerides The labeled triglycerides were synthesized from glycerol and fatty acid as described by Borgstrom and Jordan 1959 Tracer amounts of the labeled triglycerides were added to purified soybean oil and transformed to emulsion with egg lecithin and glycerol in a Logeman pressure homogenizer at 80 C The labeled emulsions contained 0 12 g of phosphatides per g glyceride A 2 5 per cent glycerol solution served as water phase

The small dose used in the first experiment was obtained by diluting the original H tripalmitin labeled emulsion with saline to a lipid content of 5 6 mg/ml as measured by weight The large dose was prepared by adding a commercial soybean oil emulsion up to a concentration of 136 mg/ml The doubly labeled emulsion used in the second experiment contained 3? mg lipid/ml The emulsions were stored at + 4 C The injected volume was 1 0 ml in all the experiments

Treatment of rats injection of fat emulsions and sampling Male Sprague Dawley rats weighing 180—230 g were used in both experiments They were first fasted for 24 hours then allowed to drink 20 (w/v) glucose in 0 45 NaCl for 12—16 hours They were given 5 ml of the same glucose solution by stomach tube two hours before the injection of the fat emulsions Under light ether anesthesia 1 0 ml of the emulsion was injected into an exposed jugular vein and the rats then allowed to regain consciousness At appropriate interval they again were anesthetized and as much blood as possible drawn from the abdominal aorta In the first experiment the liver the heart the lungs the kidneys the spleen the epididym l fat pads and a piece of the abdominal wall muscles were cut out rinsed in water blotted dry and transferred into chloroform methanol 2 1 The blood and muscle samples were weighed before being transferred to the solvent The liver was homogenized immediately in a Waring blender the other tissues at later times The remains of each rat after the tissue samples had been removed designated the carcass was frozen and stored at — 20 C

To measure the oxidation of the fatty acids of the injected fat emulsion in experiment I individual rats were killed immediately after the injection of the fat emulsion and other rats at the longest times studied These rats were homogenized *in toto* immediately and treated as previously described (Belfrage *et al* 1963)

In the second experiment only blood the liver and the spleen were sampled and treated as above

Analysis performed on the samples All tissue samples were treated as previously described (Belfrage *et al* 1963) except that the blood samples were now treated like the liver because of the larger amount of blood collected (7—9 ml)

In both experiments the liver lipids were separated into a non phospholipid fraction (NPL) and a phospholipid fraction (PL) as previously described (Olivecrona 196?a) This NPL fraction contains cholesterol esters cholesterol tr d and monoglycerides and free fatty acids

The frozen carcasses were digested in a mixture of 100 g KOH 300 ml of ethanol and 100 ml of water They were heated on boiling waterbaths overnight diluted to 1 000 ml with ethanol thoroughly stirred and duplicate aliquots taken into separatory funnels The aliquots were diluted with equal volumes of water the mixture acidified with HCl and extracted three times with petroleum ether To get good recoveries in these extractions it was found necessary to allow several hours for the phases to separate completely The extracts were washed with water and dried over anhydrous sodium sulphate The fatty acids were weighed and their radioactivity determined as for the other tissue samples The weights of the fatty acids were multiplied with a factor to get the weights of the corresponding amounts of lipid This factor was obtained in the following way samples of the lipid extracts from the rats used for the determination of oxidation of the injected emulsion were hydrolyzed and the fatty acids extracted as described above The ratio of the weight of the fatty acids to the weight of the unhydrolyzed fat was calculated in several rats A mean ratio of 1 ? was found and used as the desired factor The weight of the carcass fat from each rat calculated in this way was added to the sum of the amounts of lipid found in the tissue samples taken from the rat and the total lipid weight was thus obtained

Intralipid 20 manufactured by Vitrum AB Stockholm

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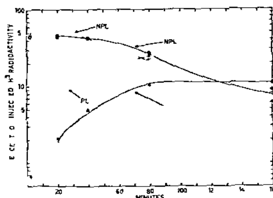


Fig 1 Radioactivity in the liver NPL and PL fractions after the intravenous injection of a fat emulsion containing H^3 palmitic acid labeled triglycerides Dotted lines 5 6 mg emulsion lipid per animal. Solid lines 136 mg emulsion lipid per animal

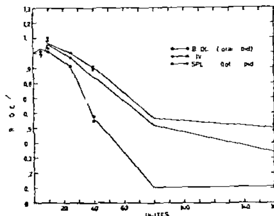


Fig 2 Ratio C^{14}/H^3 in blood lipids liver NPL and spleen lipids after the intravenous injection of 35 mg emulsion lipid containing C^{14} glycerol and H^3 palmitic acid labeled triglycerides

found in the circulation at 20 min From these data it can not be decided if the initial part of the disappearance followed a single exponential course The disappearance curves then levelled off to values of 0 7—0 8 % with both doses.

In the second experiment the ratio C^{14}/H^3 was close to unity during the first 10 min At 25 min, when only a small fraction of the injected radioactivity was still in the blood it had decreased to 0 9 It then fell rapidly to values around 0 1

Liver The maximal uptake with the small dose was 60—70 and with the large dose about 50 % The total liver radioactivity decreased appreciably after the maximum had been reached The liver NPL and PL radioactivity is shown in Fig 1 The level of radioactivity in the NPL fraction decreased rapidly with a simultaneous rise in the PL fraction At long times most of the label was found in the PL fraction

In expt 2 the liver lipids were separated into NPL and PL fractions and the ratios C^{14}/H^3 studied The level of C^{14} radioactivity in the PL fraction was too low to be accurately determined As shown in Fig 2 the ratio C^{14} H^3 in the NPL fraction was close to unity during the first 25 min and then decreased to about 0 3 These values as well

as the ratios in the blood and spleen have not been corrected for any C^{14} radioactivity in the fatty acids as described before (Olivecrona 1962 c)

Adipose tissue and muscles The values for adipose tissue radioactivity were obtained by multiplying the radioactivity in the samples studied with a factor for total adipose tissue The weight of total adipose tissue was taken as 70 % of the total lipid weight of the rat Thus two unproven assumptions were made 1 that the specific radioactivity (cpm/weight of lipid) of epididymal adipose tissue and total adipose tissue were the same and 2 that 70 % by weight of the total lipid of the rat was adipose tissue The values for the muscle radioactivity were calculated by multiplying the radioactivity in the samples studied with a factor for total muscle The latter was taken as 45 % of the body weight of the rat The specific radioactivity of the total muscle mass was thus assumed to be the same as that of the muscle sample studied (abdominal wall muscle)

As shown by the table the radioactivity in the adipose tissue showed an initial peak of 3—4 % with both doses followed by a decrease and a final slow increase Radioactivity in the muscles followed similar curves

Spleen The spleen showed a broad peak of radioactivity with a maximal uptake of about 4 % with the small dose and 13 % with the large dose Subsequently the radioactivity decreased rapidly In the second experiment the ratio C^{14}/H^{3} was above or close to unity during the first 40 min and then slowly decreased to 0 5

Other tissues Initially the lungs took up 3—5 % but the level of radioactivity rapidly decreased to values of 1 3—2 0 % Only small amounts of label were taken up by the heart and kidneys as shown in the table

Total fatty acid radioactivity recovered These values represent the sum of the radioactivity in all tissues sampled including the carcasses As shown in table 1 these values are at the longest times similar to the values obtained from the rats which were homogenized and extraced *in toto* (given in the table as whole animals)

The sum of the radioactivity in the liver the heart the kidneys the spleen and the lungs and that calculated to be in the blood the adipose tissue and the muscles is lower than the total recovery of radioactivity from the same animal It is believed that this difference as much as 25 % of the injected radioactivity in some instances is due to a combination of two factors

1 The factors used to calculate adipose tissue and muscle radioactivity gives an underestimate of the true level of radioactivity in these tissues

2 Some radioactivity is present in tissues not studied individually e g bone marrow lymph nodes etc

Discussion

The data in the first experiment revealed that the disappearance from the circulation and the tissue distribution of the fat emulsion was considerably different from that of chylomicrons used in previous experiments The rate of disappearance was higher than with comparable chylomicron doses and except for the longest times studied the sum of radioactivity in the liver and spleen was almost twice as high as in a previous chylomicron experiment (Belfrage *et al* 1963) In that experiment it was suggested that with large fat loads the liver may be relatively saturated and it is possible that a similar mechanism is also operative with the fat emulsion The spleen took up a considerably higher fraction with the large dose than with the small dose in this experiment while in the chylomicron experiment the splenic uptake was small with all doses The hepatic

uptake however showed the same inverse relationship to dosage as was found in the chylomicron experiment (Belfrage *et al* 1963)

There are several possible explanations for these differences between the fat emulsion and chylomicrons As suggested by other authors (Dole and Hamlin 1962) the distribution pattern of particulate fat might be considerably influenced by the surface properties of the particles We have noticed that the behaviour of chylomicrons which have been frozen and thawed repeatedly to destroy their physical chemical structure is similar to that of artificial fat emulsion particles i e they disappear rapidly from the circulation and are taken up to a large extent by the liver and spleen (Belfrage and Olivecrona unpublished observation) Thus chylomicrons treated in this way seem to become similar to artificial fat emulsion particles The differences in the behaviour of the fat emulsion particles and normal chylomicrons may thus probably be attributed to differences in their physical chemical structure Although it is impossible to decide the mechanism whereby the physical chemical structure influences the behaviour of the fat particles from the data of this experiment a possible explanation can be suggested Previous workers using histological techniques have shown that injected fat emulsions (Woerner 1949 Murray *et al* 1951) but not chylomicrons (Murray *et al* 1951) were initially to a large extent deposited extracellularily *e g* in the sinusoids and the capillaries of the spleen liver and lungs The lipid was deposited as large droplets indicating an agglutination and a coalescence of the fat particles At longer time intervals after the injection of the emulsion the lipid had entered into the cells of the liver and spleen while most of it had disappeared from the lungs probably flushed away by the circulating blood The large accumulations of fat in the liver and spleen after the injection of fat emulsion was partly attributed to this extracellular deposition (Murray *et al* 1951) Our data are consistent with this view although they do not give any direct evidence From the data for the small dose in our first experiment it is obvious that the increase in the liver and spleen radioactivity with time was larger than the simultaneous decrease of the blood radioactivity This can only be explained by a transport of label from other tissues to the liver and spleen The lungs and the muscles showed high initial levels of radioactivity which decreased rapidly It is possible that a large fraction of the fat emulsion appearing in these tissues was initially deposited extracellularily as described above and then rapidly flushed away by the blood which would account for the decreased radioactivity of these tissues

Although the fat emulsion disappeared more rapidly from the circulation and showed a different morphological distribution from chylomicrons the fat emulsion triglyceride seemed to be metabolized in the same way as the chylomicron triglyceride In previous experiments it has been shown that during the disappearance from the circulation of the main part of injected chylomicrons labeled with C^{14} glycerol and H^3 palmitic acid the ratios C^{14}/H^3 in the blood and liver are close to the ratio in the injected lipids (Borgstrom and Jordan 1959 Olivecrona 1962 c) This has been taken as evidence that the chylomicron triglycerides were not extensively hydrolyzed intravasally before the uptake into the tissues However if the triglycerids were partially hydrolyzed and the lower glycerides and liberated fatty acids taken up at the same rate then the ratio C^{14}/H^3 would not be much changed from that in the injected triglycerides Nevertheless, extensive intravasal lipolysis is improbable for another reason After injection of albumin bound free fatty acids approximately equal amounts of label are found in the liver NPL and PL fractions (Olivecrona 1962 a) but after the injection of chylomicrons label is found almost exclusively in the liver NPL fraction (Olivecrona 1962 b Belfrage

et al 1963) Our data (Fig 1 and 2) show that the ratio C^{14}/H^3 during the disappearance period was close to unity in the blood liver NPL and spleen and that initially label appeared almost exclusively in the liver NPL fraction Thus the fat emulsion triglyceer ides were most probably taken up into the liver and spleen without any previous extensive intravasal lipolysis

The incorporation of label into the liver PL fraction occurred at imilar rat s as those found with chylomicrons (Belfrage *et al* 1963) In a previous experiment using doubly labeled chylomicrons it was found that only fatty acids were incorporated into the liver PL fraction (Olivecrona 1962 c) The C^{14} radioactivity in the liver PL of this experiment was too low to be accurately determined However it was obvious that only a small fraction of the C^{14} radioactivity could have been incorporated into the liver PL and thus our data are consistent with the view that only small amounts of the C^{14} glycerol are incorportated into this fraction The data on the small dose of fat emulsion show that after 20 min the extrahepatic tissues increased their radioactivity This indicates a secondary retransport of fatty acids from the liver to extrahepatic tissues as has previously been shown in chylomicron experiments (Olivecrona 1962 b Belfrage *et al* 1963) The decrease of the values for total recovery and the values from the whole animals (given as total in the table) indicate an oxidation of the fatty acids of at least the same magnitude as that found in the chylomicron experiment (Belfrage *et al* 1963)

The present data thus show that in comparison to similar doses of chylomicrons an artificial fat emulsion is removed more rapidly from the circulating blood and is taken up more extensively by the liver and spleen In view of the known preferential uptake of foreign particles by the reticuloendothelial cells of the liver and spleen (Benacerraf *et al* 1957) it is possible that the high hepatic and splenic uptake of fat emulsion particles and altered chylomicrons may be related to reticulo-endothelial activity The differences between the chylomicrons and the fat emulsion particles are probably due to differences in their physical chemical structure and seem to be limited to the rate of removal from the blood and the tissue distribution The fat emulsion triglyceride as the chylomicron triglyceride is taken up without extensive intravasal hydrolysis into the liver and this is true also for the uptake into the spleen A retransport of labeled fatty acids from the liver and spleen to other tissues especially the adipose tissue and the muscles occurs in this experiment A similar retransport from the liver to the same tissues was observed in a previous chylomicron experiment The oxidation of the injected fatty acids is also of the same magnitude as that found with chylomicron fatty acids

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only one tissue sample however and thus give no information on the variation between individual animals Neither has any systematic study been made on the polyamine content in relation to age

The amido black method for the quantitative determination of spermidine and spermine permits the analysis of relatively small tissue samples as described previously (Raina 1963) The present work deals with variation between the polyamine contents in different rat tissues of both sexes and variation in a particular organ between individual animals as well as with changes in the polyamine contents in relation to age

Methods

The animals used were albino rats of the Wistar strain which had been fed *ad libitum* with a standard diet (Hankkija Oy) Analyses were made from tissues of newborn animals of male rats aged 1 3 or 9 months and of females aged 3 months

Preparation and homogenization of tissues The animals were weighed and killed by decapitation under ether anaesthesia Organs were dissected out on an ice cooled sheet of steel Care was taken to prevent evaporation before homogenization by collecting the tissue samples under cooled glass chambers The heart and various parts of the gastro intestinal tract were cut up rinsed carefully and dried gently with blotting paper After weighing the samples were homogenized with an Ultra Turrax homogenizer (Janke & Kunkel Stauffen) one part of tissue to 4—20 parts (w/v) of 0 1 N hydrochloric acid except very small parenchymatous organs which were homogenized in a Potter Elvehjem type homogenizer

Determination of spermidine and spermine In the quantitative determination of the polyamines amido black was used as the colour reagent after extraction of the amines into n butanol and subsequent paper electrophoretic separation A detailed description of the method has been published previously (Raina 1963) The minor modifications used in the present study were as follows 0 1 M citric acid sodium hydroxide buffer pH 4 3 was used for paper electrophoresis instead of the sulphosalicylic acid buffer because of some instability of the latter and 2 per cent amido black in glacial acetic acid-dist water (1 9) which remains saturated longer was used as the colour reagent

Results

Polyamine Contents in Different Tissues of Rats Aged Three Months

Table I demonstrates the polyamine contents of different tissues of male and female rats at the age of three months In general there are considerable variations between different tissues In both sexes the highest concentrations of spermidine 1 400 to 1 600 mμmoles per g wet weight were found in the thymus Large amounts of spermidine 600 to 900 mμmoles per g were further detected in the liver spleen lungs and different parts of the gastro-intestinal tract On the other hand only traces of this polyamine were found in the retroperitoneal fat As a rule no noteworthy sex differences could be observed

In general the organs rich in spermidine also contained relatively large amounts of spermine In addition the kidneys contained much spermine In most of the tissues studied the molar concentration of spermidine was higher than that of spermine as indicated by the molar ratio in Table I The only exceptions to this rule were the kidneys heart skeletal muscle and testes The livers of male rats contained somewhat less spermine than those of females which is also reflected in the molar ratio

The dispersion in the polyamine contents of a particular organ or tissue was in most cases remarkably low the standard deviations being about ± 10 per cent or less On the other hand a considerably higher dispersion was found in the data for the lungs and the tissues with a low polyamine concentration *e g* fat and skeletal muscle

TABLE I Spermidine and spermine contents of male and female rats aged three months expressed as mμmoles/g wet weight mean ± S D (standard deviation) Average weights of animals 213 g of males and 160 g of females six animals in both sex groups Spd = spermidine Sp = spermine

Organ	Sex	Spd ± S D (mμ moles/g)	Sp ± S D (mμ moles/g)	Molar ratio (Spd/Sp ± S D)
Thymus	♂	1 590 ± 246	776 ± 120	2.05 ± 0.12
	♀	1 384 ± 146	776 ± 98	1.78 ± 0.20
Liver	♂	892 ± 66	699 ± 64	1.28 ± 0.12
	♀	889 ± 58	883 ± 11	1.01 ± 0.07
Small intestine	♂	856 ± 83	487 ± 43	1.76 ± 0.11
	♀	788 ± 113	479 ± 55	1.65 ± 0.23
Spleen	♂	770 ± 46	615 ± 39	1.25 ± 0.03
	♀	786 ± 64	638 ± 50	1.23 ± 0.04
Lungs	♂	6 0 ± 166	387 ± 92	1.62 ± 0.17
	♀	761 ± 200	471 ± 95	1.62 ± 0. 5
Large intestine	♂	630 ± 107	465 ± 52	1. 3 ± 0.16
	♀	728 ± 67	520 ± 38	1.40 ± 0.12
Stomach	♂	616 ± 62	541 ± 57	1.14 ± 0.09
	♀	690 ± 94	578 ± 75	1.20 ± 0.11
Brain	♂	480 ± 78	283 ± 12	1.70 ± 0.12
	♀	452 ± 27	269 ± 24	1.68 ± 0.18
Kidneys	♂	351 ± 40	667 ± 81	0.53 ± 0.07
	♀	404 ± 46	690 ± 34	0. 9 ± 0.04
Heart	♂	232 ± 20	278 ± 19	0.83 ± 0.12
		249 ± 26	325 ± 23	0.77 ± 0.07
Skeletal muscle	♂	109 ± 13	231 ± 30	0.47 ± 0.03
		114 ± 27	209 ± 43	0.55 ± 0.08
Fat retroper	♂	80 ± 9	68 ± 16	1.18 ± 0.27
		79 ± 17	9 ± 15	1.31 ± 0.27
Seminal vesicles	♂	2 0 ± 6	160 ± 24	1.43 ± 0.31
Testes		220 ± 23	500 ± 44	0.44 ± 0.04
Uterus[1]	–	483 – 522	306 – 310	1.57 – 1.67

[1] Two pooled samples 3 organs in each.

TABLE II Spermidine and spermine contents in tissues of newborn rats (both sexes) and of male rats aged 1 3 or 9 months expressed as mμ moles/g wet weight mean ± S D Average weights of animals newborn (age group 0) 5 g at 1 month 61 g at 3 months 213 g and at 9 months 328 g Each value represents a mean of at least six samples unless otherwise indicated

Organ	Age (months)	Spd ± S D (mμmoles/g)	Sp ± S D (mμmoles/g)
Liver	0	1 976±94	438±44
	1	1 296±96	664±32
	3	89[illegible]±66	699±64
	9	576±91	68[illegible]±71
Thymus	0	1 943	603
	1	1 795±180	771±141
	3	1 590±246	776±120
	9	1 385±310	753±145
Spleen	[a]0	1 515	567
	1	1 225±127	823±40
	3	770±46	615±39
	9	756±100	735±56
Kidneys	0	1 072±54	662±60
	1	524±33	875±38
	3	351±40	667±81
	9	260±13	636±39
Skeletal muscle	0	616±42	295±59
	1	188±10	276±29
	3	109±13	231±30
	9	10±10	97±12
Brain	0	579±63	441±[illegible]9
	1	405±68	314±33
	3	480±28	[illegible]83±12
	9	382±7[illegible]	184±32

[a] Two pooled samples 10 organs in each

Changes in Polyamine Concentrations in Relation to Age

The mean polyamine concentrations of six different tissues of rats aged 0 (newborn) 1 3 or 9 months are presented in Table II The tissues studied included liver thymus spleen kidney skeletal muscle and brain Except for the newborn animals whose sex was not determined the different age groups consisted merely of male rats

In the newborn animals every tissue studied contained more spermidine than spermine calculated on a molar basis. The highest concentrations of spermidine over 1,900 mμmoles per g were found in the liver and thymus The values obtained from

TABLE I Spermidine and spermine contents of male and female rats aged three months expressed as mμmoles/g wet weight mean±S D (standard deviation) Average weights of animals 213 g of males and 160 g of females six animals in both sex groups Spd = spermidine Sp = spermine

Organ	Sex	Spd±S D (mμmoles/g)	Sp±S D (mμmoles/g)	Molar ratio (Spd/Sp±S D)
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	♀	1 384±146	776±98	1 78±0 20
Liver	♂	892±66	699±64	1 28±0 12
	♀	889±58	883±11	1 01±0 07
Small intestine	♂	856±83	487±43	1 76±0 11
	♀	788±113	479±55	1 65±0 23
Spleen	♂	770±46	615±39	1 25±0 03
	♀	786±64	638±[illegible]0	1 23±0 04
Lungs	♂	630±166	387±92	1 69±0 17
	♀	761±200	471±95	1 62±0 25
Large intestine	♂	630±107	465±52	1 33±0 16
	♀	728±67	520±38	1 40±0 12
Stomach	♂	616±62	541±57	1 14±0 09
	♀	690±94	578±75	1 20±0 11
Brain	♂	480±28	283±12	1 70±0 12
	♀	452±27	269±24	1 68±0 18
Kidneys	♂	351±40	667±81	0 53±0 07
	♀	404±46	690±34	0 59±0 04
Heart	♂	232±20	278±19	0 83±0 12
	♀	249±26	325±23	0 77±0 07
Skeletal muscle	♂	109±13	231±30	0 47±0 03
	♀	114±27	209±43	0 55±0 08
Fat retroper	♂	80±9	68±16	1 18±0 27
	♀	79±17	59±15	1 34±0 27
Seminal vesicles	♂	230±26	160±24	1 43±0 31
Testes	♂	220±23	500±44	0 44±0 04
Uterus[1]	♀	483–522	306–310	1 57–1 67

[1] Two pooled samples 3 organs in each

show minor changes during growth agree fairly well whereas the spermidine contents reported by Rosenthal and Tabor are for many tissues higher than those presented here

The high concentration of spermidine in the newborn animals and subsequent decrease with age was a uniforme feature In contrast the spermine content which in the thymus liver spleen and kidneys was appreciably lower at birth compared with spermidine showed some increase during the first month of life Thereafter spermine also fell gradually with aging Indirect evidence obtained in isotope experiments with chick embryos (Raina 1963) and with young rats (Raina to be published) supports the assumption that spermidine acts as a precursor in spermine synthesis Further — if one assumes this hypothesis to be correct — it seems obvious that the turnover of spermidine to spermine is rather slow at least in chick embryos and in young rats As shown above in newborn rats spermidine constitutes the major fraction of the polyamines in the six tissues studied and also in the whole animal (Rosenthal and Tabor 1956) In general a high spermidine/spermine ratio would be expected in rapidly growing organism In agreement with this there are some other findings *e g* a high molar ratio varying from 3 to 7 in some experimental tumours of mouse including lymphoma sarcoma and hepatoma (Rosenthal and Tabor 1956) Unfortunately very little is known at present time about the various factors affecting this ratio *e g* the concentration of the precursors and enzymes participating in the biosynthesis of the polyamines as well as the mode and rapidity with which they are eliminated by animal tissues All in all further evidence is needed to evaluate the significance of the spermidine/spermine ratio

In spite of the fact that polyamines are widely distributed in animal tissues and in microorganisms the possible physiological role of these substances is difficult to evaluate at present Various stabilizing effects which they exert on nucleic acids and ribosomes (*e g* Tabor 1962 Cohen and Lichtenstein 1960) their occurrence in ribosomes (Cohen and Lichtenstein 1960) their ability to stimulate amino acid incorporation into ribonucleoprotein particles (*e g* Hershko Amoz and Mager 1961) seem to suggest a relation to nucleic acid and protein synthesis Further it has been noted that in the chick embryo ribonucleic acid and the polyamines show similar changes during development (Raina 1963) If the changes in the DNA and RNA concentrations of various rat tissues in relation to age (Fujii and Koyama 1962 Oliver *et al* 1962 Devi *et al* 1963) are compared with those found in the polyamines such a correlation is not so clear but a certain parallelism with the changes in nucleic acids especially in the case of spermidine can be seen *i e* a decrease with increasing age of the animals Our present knowledge of the metabolism of the polyamines is too scanty to allow any definite conclusion to be drawn from this correlation

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Acta physiol scand 1964 62 359—363

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Effect of Tyramine, Reserpine and Cocaine on the Noradrenaline Release and Uptake of the Perfused Rabbit Kidney

By

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Abstract

Inouye A and I Tanaka *Effect of tyramine reserpine and co ine on the norad enaline rel ase and uptake of the perfus d kidney* Acta physiol. scand 1964 *62* 359—363 Perfusion of isolated rabbit k dn ys for 120—150 min vith Tyrode solution containing 0 05 per cent ipronia de caused no change of the normal noradrenaline (NA) content Addition of NA to the perfusion fluid caused an upt ke of NA approximately proportional to the NA concentration The majo part of the NA retained as emoved by subsequent perfus on th NA f ee Tyrode solution Perfusion with tyramine 10 4 M removed a large part of the renal NA even in the presence of NA n the perfus on fluid Reserp ne 4 × 10 M did not significantly elease NA or alt the renal NA conte it but diminished the NA uptake from a solution containing NA Cocaine caused a moderate release of NA and p rtially p evented the NA uptake wh le t hardly caused any loss in the renal NA content suggest ng some NA resynthesis

The influence of various drugs on the uptake and release of noradrenaline in organs has been studied in later years in animals in vivo (Hertting Axelrod and Whitby 1961) in isolated perfused organs (Muscholl 1960 a b) in organ slices (Brodie *et al* 1960) and in isolated storage granules (Euler and Lishajko 1961 1962)

In the present study the isolated perfused rabbit kidney was used and the uptake of noradrenaline (NA) under various conditions measured In addition the effects of tyra mine reserpine and cocaine on the amounts of NA released into the perfusion flu d and on the amounts of NA remaining in the organs were studied

Experimental

In the perfusion expe ments th right kidney was removed from rabb ts under u ethane anesthes a (1 0—1 5 g kg) w ghed cut nto p eces and homogen zed with a Turrax hom gen zer in 10 p c nt tr hlorac tic cid solut on The NA and adrenal n (A) content of the extract was d t rm ned accord ng to Euler and Lish jko (1961) After l gat on of the renal artery the left k dney was also taken out we ghed and perfused at 38 C w th Tyr de solution containing 0 05 per cent pron a ide in ord r to inh b t the act on of monoam ne o dase Perfusion was made through a polyethylene tub ng placed in the enal artery

TABLE I Noradrenaline (NA) and adrenaline (A) content of right and left rabbit kidney Mean and S E M 8 experiments

	Right kidney			Left kidney			Per cent diff in NA (paired comparison)
	Weight (g)	NA (μg/g)	A (μg/g)	Weight (g)	NA (μg/g)	A (μg/g)	
Mean	7 65	0 28	0 015	7 90	0 28	0 017	2 26
S E M		± 0 037			± 0 032		± 0 93

TABLE II Gain or loss in NA content of perfused (left) kidney and in perfusate after perfusion *with iproniazide — Tyrode* solution containing tyramine reserpine or cocaine compared with unperfused control

Number of expts	Drug added to perfusion fluid	Perfusion time (min)	Renal NA content (μg/g)			NA in perfusate (μg/g kidney)	Mean net gain or loss (μg/g kidney)
			Control	Perf	Diff		
4	—	120–150	0 33 (0 18–0 43)	0 34 (0 19–0 44)	<0 05	<0 05	<0 05
3	Tyramine 10^{-4} M	120	0 35 (0 23–0 52)	0 18 (0 10–0 31)	0 17 (0 13–0 21)	0 15 (0 12–0 17)	<0 05
3	Reserpine 4×10^{-6} M	100–180	0 28 (0 22–0 35)	0 25 (0 19–0 32)	<0 05	<0 05	<0 05
3	Cocaine 10^{-4} M	120	0 24 (0 19–0 30)	0 24 (0 20–0 29)	<0 05	0 07 (0 03 0 10)	0 07 (0 05–0 11)

After the perfusion was started the perfusate through the vein and other routes as collected every 20—30 min and its volume measured NA in various concentrations tyramine (*10⁻⁴ M*) reserpine (4 × 10 M) or cocaine (10⁻⁴ M) were added to the *perfusion fluid as indicated*

The flow rate of perfusion fluid was adjusted to 5—12 ml/min by a clamp on the tubing from the reservoir

To the effluent 1/20—1/10 volume of 10 trichloracetic acid solution was added After filtration if necessary the catecholamines were determined in *20—100 ml of the perfusate*

After perfusion the kidney was blotted dry with a filter paper to eliminate any adhering NA-*containing* solution weighed and extracted *with 10 trichloracetic* acid The NA concentration in the perfusing fluid was determined on samples taken in the beginning and the end of perfusion their average taken as the effective NA concentration NA *values of the kid*ney are given as the base (uncorrected for about 20 per cent loss) and refer to the weight before perfusion

The Tyrode solution used contained in 1 l tre NaCl 8 g KCl 2 5 g CaCl 2.5 g MgCl 6H O 2.5 g NaHCO 2 5 g NaH PO H O 1 25 g dextrose 1 0 g and iproniazide 0 5 g It was aerated with 6 CO + 94 O giving a final pH value of 6 9—7 1

Three types of perfusion experiments were performed in one series the total amount of NA in the collected effluent and the changes in NA content of the perfused kidney were estimated after perfusion for 100—180 min with drugs n the second series the kidney was perfused with Tyrode containing NA and drugs and in the third series this perfusion was followed by NA f ee Tyrode solution for about one hour and the NA content of the perfused kidney deter mined

Paired comparison of catecholamine content of rabbit kidneys

In 8 rabbits the NA and A content was determined separately in the right and left kidney (Table I) In no case did the difference exceed 14 % its mean and standard deviation being 2 3 + 2 5 % (S D) when compared in pairs Differences in NA content between control and after perfusion beyond 15 % were considered statistically signifi cant in the experiments with drugs added to the Tyrode solution

Effect of perfusion with iproniazide Tyrode solution on the catecholamine content of rabbit s kidney

The normal NA content of 53 rabbit kidneys used as controls fell within the range of 0 17—0 52 μg/g their average being 0 30 $\pm$ 0 03 μg/g (M $\pm$ S E M) which is in good agreement with previous reports (Rehn 1958) In 4 perfusion expts the NA con tent was measured in the unperfused control (right kidney) and in the left kidney after perfusion for 120—150 min with iproniazide Tyrode solution As shown in Table II the mean difference in the NA content of the two kidneys was less than 0 05 μg/g kidney

Pe fusion with iproma ide Tyrode solution containing tyramine reserpine or cocaine

While perfusion with iproniazide Tyrode solution for 120—150 min left the NA content unchanged addition of tyramine 10⁻ M caused a strong NA depletion (Table II) and also a marked increase of NA in the perfusate If the presence of iproniazide in the Tyrode solution has interfered with the release of NA from the organ (Axelrod Hertting and Patrick 1961) it clearly did not prevent the depleting effect of tyramine

The change in NA content of the kidney as well as the NA output into the effluent was insignificant during perfusion with Tyrode solution to which reserpine had been added Cocaine (10 M) caused a moderate NA increase in the perfusate while the renal NA content remained unaltered under the effect of this drug suggesting some repletion of renal NA during the perfusion

Perfusion with NA-containing Tyrode solution with and without drugs

After perfusion with NA-containing solutions for 90—120 min an increase in renal NA content was found (Fig 1) As seen in the figure the highest uptake of a given concentra tion of NA in the perfusion fluid occurs with NA alone while it is considerably less in the presence of the drugs studied and particularly so for tyramine

Perfusion with NA-containing Tyrode solution with and without drugs followed by Tyrode perfusion

When perfusion with NA-containing solution for 90 min was followed by 60 min perfusion with Tyrode solution the greatest part of the NA accumulated in the tissues is washed out However as shown in Fig 2 concentrations of NA beyond 0 10 μg/ml

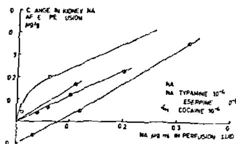


Fig 1 Isolated rabbit kidney perfused 90—120 min with Tyrode solution containing 0 05 per cent iproniazide and varying concentrations of NA Influence of tyramine reserpine and cocaine added to the perfusion fluid on the uptake of NA in the kidney

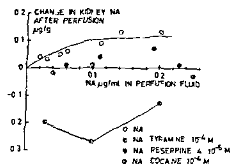


Fig 2 Isolated rabbit kidney perfused for 90 min as in Fig 1 followed by 60 min perfusion with Tyrode + iproniazide

in the perfusion fluid resulted in a net increase in NA content of 0 08—0 12 μg/g in the kidney probably representing a true uptake When NA was perfused together with reserpine (4×10^{-6} M) or cocaine (10^{-4} M) no definite permanent uptake was observed, the change in NA content being (with one exception) within the range of ± 0 05 μg/g even after perfusion with concentrations of 0 20—0 25 μg/ml of NA Tyramine however was found to diminish the renal NA content strongly (0 14—0 27 μg/g in 3 expts)

The mean uptake of NA found in this series after washing suggests that the NA taken up is bound to tissues in such a form that it is readily removed by washing with Tyrode solution. Also it appears that reserpine and cocaine inhibited this uptake Whether tyramine inhibits the uptake in addition to its NA-depleting action cannot be decided by the present experiments

Discussion

In our experiments perfusion of the kidney with NA-containing Tyrode solution followed by washing with NA free solution caused a small but definite increase in the renal NA content while NA together with reserpine or cocaine caused practically no change in the organ content after washing As shown by Wegmann and Kako (1961) a large part of NA injected to the dog is recovered in the soluble fraction of the heart tissue while the amount found in the particulate bound fraction was small Since most of the NA taken up after perfusion with NA can be *washed out with Tyrode solution in contrast* to the normally occurring NA it is assumed that the latter occurs in a bound form *presumably* in part in particulate elements The remaining increase in NA content after perfusion with NA and subsequent perfusion with Tyrode may therefore represent a "true uptake in a bound form

Tyramine releases NA from organs not only in vivo (Stjarne 1961) and *in the perfused organ* (Lindmar and Muscholl 1961 Angelakos and Torchiana 1963) but also from

isolated storage granules (Schumann 1960 Euler and Lishajko 1960) as well as from the particles in the intact cells (Iversen and Whitby 1963) Our results support the concept that tyramine displaces noradrenaline from the stores in the organ and also show that the endogenous NA is not recovered during the subsequent perfusion period with Tyrode solution

In the present experiments reserpine in the concentration used had no direct releasing action on the endogenous NA in the kidney This is in agreement with the observations of Euler and Lishajko (1961) that only high concentrations of reserpine had a direct releasing effect while lower concentrations inhibited the amine release from isolated storage granules The preventing action of reserpine on amine uptake by the tissues (Brodie *et al* 1960 Muscholl 1960 b) especially by the particles in the intact cells of the heart (Campos and Shideman 1962) is in agreement with the lowered NA uptake found The inhibitory action of cocaine on the uptake of NA is in harmony with the findings of Muscholl (1960 a) on the perfused heart

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From the Neurophysiological Laboratory Anatomical Institute University of Oslo Norway

On the Silent Period and Golgi Tendon Organs of the Soleus Muscle of the Cat

By

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Abstract

Jansen J K S and Rudjord T *On the silent period and Golgi tendon organs of the soleus muscle of the cat* Acta physiol scand 1964 *62* 364—379 The silent period in the soleus during twitch contractions has been studied in intercollicularly decerebrated cats The duration of the silent period was independent of the initial tension in the muscle and remained constant in spite of large variations in the twitch tension with springs of varying compliances inserted in series with the muscle. The responses of 42 tendon organs of the soleus to twitch contractions passive stretch and fused tetanic contractions have been determined in Nembutal anaesthetized cats The majority of the tendon organs had appreciably lower thresholds to active contraction than to passive stretch of the muscle They were all excited by isometric twitches developing less than 160 g tension *There was an approximately linear relationship between the discharge* frequency of the tendon organs and the tension in the muscle during isometric graded fused tetanic contractions of the muscle The slope of this relationship varied between 2 and 18 imp/sec/100 g It is concluded that the duration of the silent period in the decerebrate cat is primarily determined by the pause in the discharge of the primary endings of the muscle spindle during the twitch

The interruption of the electrical activity of a muscle with intact innervation during a superimposed twitch or tendon jerk (the silent period) has been investigated extensively since its discovery by Hoffmann (1919) (for a review see Granit 1955) The object of these investigations has been to elucidate the reflex effects of the afferent impulses from the various sense organs in the muscle and certain functional properties of the motoneurones involved It is generally agreed that three different spinal mechanisms will contribute to a varying degree to the silent period

1 The recurrent inhibition of motoneuronal activity by means of the recurrent collaterals and Renshaw cells (Renshaw 1946 Eccles Fatt and Koketsu 1954) The silent period ascribed to synchronized activity of slowly discharging *motoneurones* (Hoff et al 1934) is presumably partly due to recurrent inhibition The time course indicates that the effect of such inhibition is most pronounced during the early part of the silent period (Eccles Fatt and Koketsu 1954 Merton 1951)

2 The pause in excitatory Ia afferent impulses from the muscle during its contraction (Fulton and Pi Suñer 1928 Matthews 1933 Merton 1951)

3 The inhibition due to activation of the Golgi tendon organs

It was emphasized by Merton (1951) that the duration of the silent period is of particular importance from the point of view of the control of movement From various observations on the silent period in adductor pollicis muscle during steady voluntary activity Merton concluded that the re-excitation of the muscle spindle receptors by their extension during the falling phase of the twitch contraction was the main factor causing resumption of firing in the motoneurones after the silent period However recently the silent period during voluntary activity has been re-examined by Hufschmidt (1960) By comparing silent periods in the biceps muscle with and without simultaneous shortening Hufschmidt reached the conclusion that the silent period is due to autogenetic inhibition by afferent impulses from the Golgi tendon organs

This discrepancy prompted the present re investigations of the silent period in the decerebrate cat It was felt that a comparison of the silent period during isometric and isotonic twitches would differentiate between the effects of the spindle pause and the tendon organ inhibition In addition it appeared desirable to study the behaviour of the Golgi tendon organs under comparable conditions These sense organs have received comparatively little attention since their functional identification by Matthews (1933)

Methods

The experiments on the silent period were performed on four cats decerebrated by intercollicular section after ligation of the carotid arteries These cats all had sustained stretch reflexes in the soleus The hind leg was partly denervated by section of the lateral and medial popliteal nerves except for the branch to the lateral head of gastrocnemius and soleus This nerve was dissected through the lateral gastrocnemius and all side branches to this muscle were cut The soleus tendon was isolated from the rest of the Achilles tendon This initial dissection and the subsequent decerebration were performed under ether anaesthesia The cat was then placed in a rigid frame and the hind leg immobilized by pins in the iliac crests and the proximal and distal ends of the tibia The soleus tendon was connected to an isometric myograph with an RCA 5734 valve as transducing element. Nearly isotonic conditions were obtained by the insertion of a weak spring between the muscle and the myograph. Stretch reflexes could then be elicited by various degrees of extension of the muscle The tension on the muscle was displayed on one beam of a Tetronix 502 oscilloscope The electromyogram of the soleus was recorded between two unisolated needles one in the muscle belly and the other in tendon and displayed on the second beam of the oscilloscope

Twitches in the muscle were elicited every second by single shock stimulation of the nerve of α maximal intensity This produced silent periods in the stretch induced electrical background activity of the muscle The oscilloscope sweep was synchronized with the stimulus and usually each frame of the film was exposed by five superimposed sweeps The sweep speed was 50 msec/cm and the duration of the silent period was measured from the beginning of the direct electrical response of the muscle to the first activity appearing after the twitch The duration of the silent period could then be measured on the film with an accuracy of about $\pm$ 3 msec

The Golgi tendon organs were studied in six cats kept lightly anaesthetized by intravenous injections of Nembutal The hind leg was denervated except for the nerve to soleus and the soleus tendon was isolated After a lumbar laminectomy the dorsal and ventral roots L VII and S I were cut proximally The dorsal roots were divided into fine filaments until only one active unit was present This was identified as a Golgi tendon organ in soleus by its behaviour during isometric twitch contractions of the muscle and by the conduction velocity of the afferent fibre determined by electrical stimulation of the nerve near the muscle (Fig 3) While searching for tendon organs, the muscle was kept twitching at one sec intervals. The

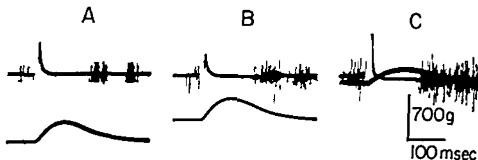


Fig 1 Silent periods at various initial tensions Each frame consists of five superimposed sweeps Electromyogram above tension record below α maximal twitches to stimulation of muscle nerve every 2 sec in all instances Initial tension in A 120 g B 470 g C 1 000 g

nerve impulses recorded from the dorsal root filaments were fed through a preamplifier (Tectronix 122) and displayed on the oscilloscope According to the requirements of the experiment the time base of display was provided either by the horizontal sweep of the oscilloscope or by the movement of the film The speed of the film was then 10 cm/sec

The discharge pattern of the tendon organs was studied during linear extension of the muscle up to a position corresponding to full physiological extension The puller consisted of a hydraulic system with a moving piston that was connected to the muscle through the isometric myograph The movement of the puller was controlled by electromagnetic valves and could be stopped at any degree of extension The rate of movement of the piston was kept at 18 mm/sec in all experiments Twitch contractions of the muscle were elicited by single shock stimulation of the muscle nerve Fused tetanic contractions were produced by stimulation of the peripheral ends of ventral roots L VII and S I at a frequency of 40/sec and intensities between threshold and α maximal Usually tetani of 2 sec duration were elicited every 10 sec

During the experiment the rectal temperature was kept between 37 and 38 C All the exposed nerves and muscles were kept covered by heated and O_2 equilibrated mineral oil in pools produced by the skin flaps

The frequency of tendon organ discharge was determined by counting the number of impulses during a 0 1 sec period In the present steady state experiments the discharges of the tendon organs were usually remarkably regular The only exceptions to this was observed when a strong ventral root tetani produced early discharges in the afferent nerve fibre Such observations have been excluded from the material

Results

A *The silent period*

In the decerebrate cats the duration of the silent period during a twitch contraction varied considerably in different preparations (80—200 msec) However in any one preparation, the duration of the silent period as a rule remained fairly constant In one cat a *spontaneous reduction in the duration of the silent period from* 170 to 100 msec was observed This was accompanied by a marked increase in the stretch reflex of the soleus and was probably due to additional cerebral damage at the site of decerebration On the whole however the duration of the silent period remained constant as long as the animal remained in a stable condition Therefore the duration of the silent period in the decerebrate cat provides a stable index of the changes in activity of spinal motoneurones and can be used experimentally to deduce the cause of such changes as indeed has been done by a number of investigators

Effect of initial tension The duration of the silent period was not appreciably changed even by large variations in the initial tension A typical experiment is shown in Fig 1

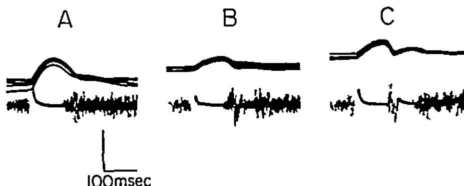


Fig 2 Silent periods with various degrees of muscle shortening A isometric twitches initial tension 700—850 g B spring of 0 025 mm/g compliance in series with the muscle Initial tension 700 g C spring of 0 18 mm/g compliance in series with the muscle Initial tension 350 g α maximal twitches to muscle nerve stimulation in all instances Each record consists of five superimposed sweeps All from the same experiment Myograph sensitivity changed so that vertical calibration bar of A corresponds to 700 g in A 175 g in B and 35 g in C

In the first records (A) the initial tension was as low as 120 g and only a few motoneurones were firing In B and C the initial tension was increased to 360 and 870 g respectively and an increasing number of motoneurones were recruited The shock to the soleus nerve was identical in all three instances and of α maximal intensity In spite of considerable increase in resting tension and the corresponding increase in excitation of the soleus motoneurones the duration of the silent period was very much the same approximately 150 msec

Considering now the three reflex mechanisms of importance for the determination of the duration of the silent period the first namely the recurrent inhibition following the antidromic volley appears an unlikely explanation of the observation recorded in Fig 1 Firstly a silent period of 150 msec is considerably longer than the usual duration of recurrent inhibition (Eccles *et al* 1954) The antidromic volley of Fig 1 was the same in all instances Taking the temporal decay of recurrent inhibition (Eccles *et al* 1954) into account one would expect a shorter silent period with the more powerful excitation of the motoneurones at increasing extensions This is also in harmony with the observations made on silent periods in deafferented muscles (Hoff *et al* 1934 Holmgren and Merton 1954)

Without knowledge of the exact behaviour of the primary endings of the muscle spindles and the Golgi tendon organs the observations shown in Fig 1 do not provide critical information as to whether the spindle pause during the twitch or the autogenetic tendon organ inhibition are mainly responsible for the observed silent period If the inhibitory effect of the Golgi tendon organ impulses as the most important factor it would have to be very accurately matched with the increasing excitation to secure the observed constancy of the duration of the silent period

Comparison of isotonic and isometric twitches The comparison of the silent periods during isometric twitches and twitches with appreciable shortening provides additional evidence on the mechanism of the silent period Records from such an experiment are shown in Fig 2 The isometric silent period had a duration of 100 msec Fig 2A)

and the initial tension varied between 650 and 850 g *Under these conditions the* maximal shock to the motor nerve caused a twitch contraction of about 400 g additional tension In Fig 2B a spring with a compliance of 0 025 mm/g was inserted between the myograph and the muscle The initial tension was in this case about 700 g and now an α maximal twitch caused an increase in tension of 40 g only In Fig 2C a weak spring with a compliance of 0 18 mm/g was inserted in series with the muscle The initial tension was 350 g and the α maximal twitch now caused only 14 g of additional tension The striking observation of Fig 2 is that the duration of the silent period remained practically unchanged at 100 msec in spite of the large reduction in twitch tension from 400 g under isometric to 14 g under practically isotonic conditions Comparable observations were made in all four cats

An additional observation of interest can be made from the records in Fig 2 Considering the resumption of firing of the motoneurones after the silent period there are marked differences between the isometric (A) and the isotonic (B and C) conditions In A the motoneurones start firing at approximately the same level as before the silent period whereas in B and C in which there was considerable shortening of the muscle during the twitch a marked overshoot in the electrical activity ensued immediately after the silent period With the weakest spring (Fig 2C) this caused a second shortening of the muscle with an accompanying silence in the electromyogram A moderate amount of overshoot certainly may occur even under isometric conditions but a more marked overshoot has been a constant feature in our experiments when shortening takes place during the twitch

Considering this observation from the point of view of the neural mechanisms of the silent period it is clear that the antidromic volley following the α maximal shock in Fig 2 will be the same whether the muscle is allowed to shorten during the twitch or not The considerable difference in the firing pattern in the two situations must therefore be due to afferent impulses from muscle receptors changing the firing pattern of the motoneurones As the overshoot in the electromyogram after the isotonic twitch occurs at approximately the same time as the resumption of firing after the isometric twitch it appears less likely that the recurrent inhibition under these experimental conditions is of primary importance for determining the duration of the silent period

Since the work of Matthews (1933) the Golgi tendon organs are generally regarded as slowly adapting receptors signalling the degree of tension in the muscle On this background the observations of Fig 2 have some bearing on the relative importance of the afferent impulses from Golgi tendon organs and the spindle pause for the duration of the silent period The constancy of the duration of the silent period in spite of the large changes in the tension developed during the twitch makes it unlikely that the inhibition from the Golgi tendon organs is an important factor in this respect

On the other hand the pause in discharges from the primary endings of the muscle spindles is determined by the time course of the twitch contraction and this is not markedly different in isometric and isotonic twitches It is known however and appears from the records of Fig 2 that the peak time of isotonic twitches (80 msec) is longer than that of isometric twitches (70 msec) With the constant duration of the silent period this must mean that the motoneurones resume firing earlier on *the falling* phase of isotonic twitches This is indeed apparent in Fig 2 This observation can be related to the considerable dynamic sensitivity of the primary receptors of the muscle spindles (Matthews 1933 Cooper 1961) The rate of reextension of the muscle will be greater following an isotonic twitch and accordingly it will evoke a more violent

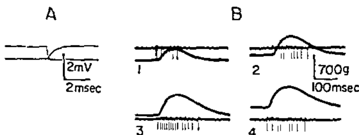


Fig 3 Identification of a Golgi tendon organ A Action potential in dorsal root filament to stimulation of the nerve near the muscle B Discharge from the tendon organ during maximal isometric twitches Initial tension in 1 is 0 g in 2 140 g in 3 395 g and in 4 640 g

discharge from the primary endings (Granit and van der Meulen 1962) Thus the conclusion is reached that under the present experimental conditions the pause in discharges from the primary endings of the muscle spindle during a twitch is the most important factor determining the duration of the concomitant silence in the electromyogram

B *Properties of the Golgi tendon organs*

Our original intension was simply to study the behaviour of the Golgi tendon organs under conditions comparable to those used in the study of the silent period. But the unexpected observation of the large difference in threshold for active contraction and passive stretch seemed to make a more extensive study desirable

The method of identification and certain characteristics of the discharges of Golgi tendon organs appear from Fig 3 The conduction velocity of their afferent fibres was always within the group I range (Fig 3A) The 42 tendon organs of the present study had afferent fibres with conduction velocities of 79—116 m sec in agreement with the observations of Hunt (1954) The typical discharge pattern of a Golgi tendon organ during an isometric twitch appears from Fig 3B Characteristically the frequency of firing is greatest on the rising phase of the twitch and the maximum is reached before the peak tension This applies to all of our observations on Golgi tendon organs and indicates that they respond to the rate of increase in tension as well as tension itself The same observation can be made during passive extension of the muscle (see below) and in the records published by Hunt and Kuffler (1951) A further point can be seen in Fig 3B With increasing initial tension there is an appreciable increase in response only as long as the active twitch tension increases with muscle extension In spite of the considerable initial tension in the last record (Fig 3B 4) there was no discharge before the active contraction Thus there appears to be a marked difference between the responsiveness of Golgi tendon organs to active contraction and to passive extension This point will be further elaborated below

Passive stretch As described by Matthews (1933) the discharge frequency of most Golgi tendon organs increases with increase in tension during passive extension of the muscle Matthews also pointed out that there is a considerable difference in threshold between different Golgi tendon organs This appears in the records of Fig 4 which display the discharge pattern of two different Golgi tendon organs during a linear stretch of 18 mm/sec up to full physiological extension of the muscle The tendon

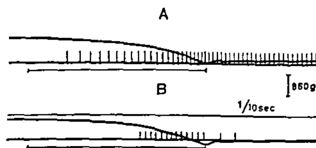


Fig 4 Discharges of two different Golgi tendon organs (A and B) in the same muscle to passive linear stretch Tension record above action potentials below Duration of dynamic stretch indicated by the line below each record The muscle was kept at the terminal length which correspond approximately to full physiological extension Velocity of stretch about 18 mm/sec

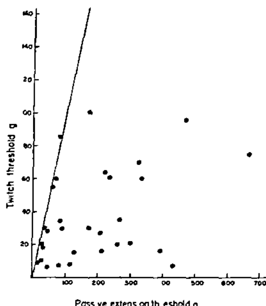


Fig 5 Scatter diagram f observations of threshold to passive stretch (abscissa) and isometric twitch contraction (ordinate) Each point represents the observations of one unit The open circles to the right indicate units that did not discharge during the passive stretch Straight line y = x drawn to indicate difference in scales on abscissa and ordinate

organ of Fig 4A started to discharge at a tension of 30 g Characteristically the discharge frequency was greater at the end of the dynamic stretch than during the following maintained stretch This is usually so even if the tension overshoot due to muscular viscosity is disregarded and the dynamic and static firing frequencies are compared at comparable tensions As pointed out above this suggests that the Golgi tendon organs respond to the rate of change in tension as well as to the tension itself During the maintained extension the adaptation of the sense organ is very slow This has been measured for the 10 tendon organs observed in the last two cats of the present series These all showed a firing pattern similar to that of Fig 4A and the average decrease in frequency during the period from 0 5 to 1 5 sec of a maintained stretch at full extension was 12 9 % (range 2 9 % to 21 4 %)

In the record of Fig 4A one may incidently notice a characteristic difference in the firing pattern of a Golgi tendon organ and that described for primary receptors in the muscle spindle during a similar stretch The primary ending shows typically a prolonged discharge interval between the dynamic and static phases of the stretch (Jansen and Matthews 1962) Such a pause has never been observed in the discharge of the Golgi tendon organs One is tempted to ask whether this may be due to the existence of two different receptor areas for the primary ending one on the nuclear bag and one on the nuclear chain intrafusal fibres each perhaps responsible for the dynamic and static discharge respectively of the primary ending (cf Jansen and Matthews 1962)

The tendon organ of Fig 4B had a much higher threshold for passive extension than that of Fig 4A and in addition it did not discharge during the maintained stretch even though this was at full physiological extension of the muscle Such behaviour was frequently encountered in tendon organs with high threshold to passive stretch and indeed the same is regularly observed in low threshold tendon organs at smaller degrees of extension As appears from Fig 5 some tendon organs with even higher thresholds than that of Fig 4B were found in the present series Of the 42 tendon organs studied 12 did not give a maintained discharge at full physiological extension of the muscle the corresponding tension being 600—700 g A few of these were found to discharge tonically on an additional 1—3 mm stretch But six tendon organs were found that did not fire at all during or after passive stretch up to about 1 100 g tension On account of the possible deleterious effect of overstretch such extensions were tried only occasionally at the end of the experiments

Comparison of twitch and passive extension thresholds From records of passive extensions like those of Fig 4A and B the amount of muscular tension at the moment of the first discharge from the sense organ was measured for the whole series of Golgi tendon organs studied in this way For the same tendon organs the peak tension of the smallest isometric twitch that would cause a discharge from the receptor was determined The result of these measurements is shown in the scatter diagram of Fig 5 in which each point represents the observations on one tendon organ The threshold value for passive extension has been plotted along abscissa and that of the twitch along the ordinate It should be noted that the scales of the abscissa and ordinate differ by a factor of five It appears from Fig 5 that none of the tendon organs studied had a significantly higher threshold for twitch contraction than for passive extension The majority had an appreciable lower threshold for twitches Furthermore there is no obvious relation between the two thresholds Some of the receptors that did not fire at all during passive extensions had a fairly low threshold for twitch contractions The thresholds for passive extension ranged from 20 g to more than 1 000 g The thresholds for twitch contractions were all between 6 g and 160 g (mean value 44 g)

The fact that the Golgi tendon organs respond to the rate of change of tension as well as to the absolute level of tension makes it clear that the values of twitch threshold and passive extension threshold presented above may not be strictly comparable as the time course of the exciting force is probably very different in the two cases Even so it is hoped that the comparison may be helpful in pointing out the marked difference in the response of Golgi tendon organs to passive extension and to active contraction of the muscle

The responce to tetanic contractions It is conceivable that high threshold to passive stretch and refusal to fire during maintained extension might be due to a more rapid rate of adaptation of certain of the Golgi tendon organs However their behaviour during maintained active contractions evoked by tetanic stimulation of the appropriate

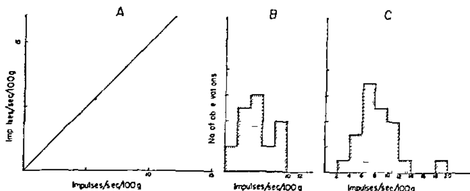


Fig 8 4 Comparison of tension frequency slopes to passive stretch (ordinate) and to tetanic contractions (abscissa) Each point represents one tendon organ I ine drawn y = x to facilitate comparison *B* Histogram of slopes to passive static extension *C* Histogram of slopes to fused tetanic contractions

behaviour is shown by all the tendon organs of Fig 7 Furthermore with initial ten sions of more than 300 g a similar increase in extrapolated thresholds was found in 17 of the 18 tendon organs for which such observations have been made On the other hand a convincing change in slope has never been observed with increase in initial tension

A less frequent feature of tendon organ behaviour appears from Fig 7B To passive extension this tendon organ had a sensitivity of approximately 3 impulses/sec/100 g (interrupted curve) On the other hand to tetanic contractions its sensitivity was more than four times as great. Characteristically the sensitivity was not influenced by the initial tension The threshold however was progressively increased In the present series of tendon organs the small difference in threshold to static extension and tetanic contraction shown in Fig 7B was somewhat exceptional Its dynamic ex tension threshold was 90 g and that of the twitch contraction 30 g (see above)

As mentioned, the change in slope shown in Fig 7B was not a very frequent obser vation in the present material Straight lines have been fitted by eye to the observations of all the 17 tendon organs for which this comparison can be made The slopes of these straight lines are presented in the scatter diagram of Fig 8A Each point repre sents one tendon organ. Its sensitivity to tetanic contractions is plotted along the ab scissa and that to static extension along the ordinate It appears from Fig 8A that none of the Golgi tendon organs had a greater sensitivity to passive than to active tension. The majority of them were nearly equally sensitive to both kinds of load Unfortunately too much reliance cannot be put on these slopes since some of the observations do not fit straight lines as nicely as those of Fig 7B C and D However a change of slope by more than a factor of two was definit ly real and thus at least four of the 17 tendon organs of Fig 8A had a greater sensitivity to active contraction than to passive extension.

Some of the tendon organs did not discharge during the passive extensions applied and the response to tetanic contractions was not examined in two of those that did Accordingly these units are not represented in Fig 8A The values of the passive extension slopes of the 19 tendon organs of the present material appear from Fig 8B



Fig 9 Localizat on in soleus of 20 tendon organs of the last two cats Tendon organs with a mainta ned discharge to full physiological stat c extension (○) tendon o gans without discharge to full static extens on (●)

The mean value of the observations is 5 impulses/sec/100 g (S D 2 8) All together the tension response curve of 25 tendon organs to tetanic contraction was determined in the present study The slopes of these curves are shown in the histogram of Fig 8C The mean value of these slopes is 8 2 impulses/sec/100 g (S D 3 0)

As mentioned above some of the Golgi tendon organs did not discharge during passive extension up to full physiological extension However their responses to tetanic contractions were indistinguishable from all the other Golgi tendon organs studied The tendon organ of Fig 7C provides an example It did not discharge at all during extension up to 600 g of passive tension Its slope to tetanic contractions was about 18 impulses/sec/100 g This was the highest sensitivity of any tendon organ of the present series With 170 g of initial tension the response curve was typically shifted to the right The sensitivity of seven other tendon organs that did not respond tonically to passive extension ranged from 9 to 2 8 impulses/sec/100 g which agrees with the range of the rest of the material

It was regularly observed (Fig 7) that the total tension response curves were shifted to the right when the contraction took place under a certain amount of initial tension In general this shift to the right corresponded approximately to the amount of initial tension (Fig 7B) so that if only active tension instead of total tension had been plotted against the response the observations would all fall on the same straight line This signifies that the tendon organs to some extent signal active tension only and disregards that due to passive extension However this is not always so With small and moderate initial tensions there is usually not a definite shift to the right suggesting that the tendon organs signal the sum of active and passi e tension An example of this is provided in Fig 7D The response to unloaded tetanic contractions and those to tetanic contractions at an initial tension of 170 g do all fall on nearly the same stra ght line On the other hand with an initial tension of 350 g the curve is definitely shifted to the right. It should be noted that the tendon organ of Fig 7B was exceptional in showing a definite increase in extrapolated threshold with only 80 g of initial tension Usually an initial tension of about 300 g was required to demonstrate the shift

Localiza n of Golgi tendon organs The s mplest explanation of the difference in response of the tendon organs to active contraction and to passive stretch of the muscle is that differently located tendon organs will be differently affected by the exciting force in the two instances Therefore in the last two experiments, the localization

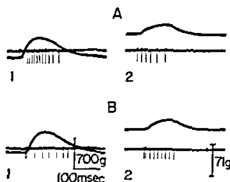


Fig 10 Comparison of tendon organ discharge during isometric and isotonic twitches *A* Tendon organ excited by passive stretch *1* Isometric twitch resting tension 140 g *2* Spring of 0 18 mm/g compliance in series with muscle Initial tension 160 g *B* Tendon organ not excited by passive stretch *1* Isom tric twitch initial tension 210 g *2* Same spring in series with muscle Initial tension 200 g All twitches were maximal for contraction Calibration in B 1 and 2 apply to both pairs of record

within the muscle of the 20 tendon organs studied was determined This can be done fairly accurately by application of pressure to the muscle Employing the pointed end of a pencil it was always possible to determine an area of less than 4 mm diameter from which a given tendon organ was most easily excited by pressure All the tendon organs gave a maintained response to such a stimulus In Fig 9 the approximate localization of these 20 tendon organs is indicated As pointed out by Swett and Eldred (1960) the tendon organs are located throughout the entire length of the muscle Each tendon organ was studied by passive stretch of the muscle up to full physiological extension and otherwise identified as shown in Fig 3 The seven tendon organs of this series that did not give a maintained discharge to passive stretch are shown by filled circles those that did by open circles It appears that the units not excited by passive stretch were all localized in the proximal part of the muscle

Comparison of tendon organ discharges during isometric and isotonic twitches Returning finally to the original problem of the tendon organ discharges during the isometric and isotonic twitches employed in the study of the silent period some characteristic records are shown in Fig 10 The upper two records (Fig 10A) illustrate the behaviour of a tendon organ that was activated by passive extension. The first record (1) shows its discharge pattern during an α maximal isometric twitch The behaviour of the sense organ during an α maximal twitch with the weak spring of 0 18 mm/g compliance in series with the muscle appears from the second record Corresponding records from a tendon organ not excited by passive extension are shown below (Fig 10B) It appears that both tendon organs do fire a number of impulses even though the increase in tension during the isotonic twitch was only 22 g On the other hand the number of impulses fired during the twitch was far greater under isometric conditions Such observations have been made for 17 tendon organs of the present series All but one of them fired some impulses during maximal twitches with the weak spring in series with the muscle and all of them fired at least twice as many impulses during an isometric twitch On the average the 17 tendon organs fired 2 8 times as many impulses during isometric as during isotonic maximal twitches Thus in view of the constant duration of the silent period in isometric and isotonic twitches in the decerebrate cat it appears less likely that the afferent impulses from the tendon organs are of primary importance for the determination of the duration of the silent period in this preparation

Discussion

As pointed out in the introduction three spinal mechanisms will contribute to the occurrence of a silent period during a twitch contraction Presumably the relative importance of each of these will vary under different experimental conditions The observations of the present study suggest that in the soleus of the decerebrate cat the duration of the silence of stretch induced electrical activity in a muscle during a twitch contraction is mainly determined by the pause in excitatory Ia impulses Considering the demonstration of Eccles and Lundberg (1959) of a tonic depression of group Ib reflex effects in the decerebrate cat the present result appears quite reasonable and should not uncritically be applied to observations from different experimental situations This may explain the entirely different conclusion concerning the mechanism of the silent period reached by Hufschmidt (1960)

It should be pointed out however that the depression of Ib reflex effects in decerebrate preparations (Eccles and Lundberg 1959) particularly concerned the Ib effects of flexor digitorum longus to the other ankle extensors and it was found to a much smaller extent between the other extensor muscles It remains for further experiments to decide whether the apparent lack of autogenetic Ib effects in the present experiments is due to descending inhibition of Ib interneurones or whether the inhibitory effect of Golgi tendon organ afferents plays a minor part only in the determination of the duration of the silent period under more physiological conditions as well

Be this as it may the present experiments provide an example of stretch reflex behaviour in the decerebrate cat that may be described formally as a length servo employing the misalignement signal from the primary endings as the negative feed back In such terms Merton (1951) interpreted his observations on the silent period during steady voluntary effort In these terms the oscillations following the isotonic silent periods of Fig 2C can be ascribed to instability on account of loop delay in the system In such systems a high degree of velocity feedback will reduce the stability For the appropriate control of the system the possibility of independent changes of the dynamic and static sensitivity of the receptors (Jansen and Matthews 1962) may be of particular importance Indeed variations in the velocity feedback in the system may partly explain the considerable variation in the duration of the silent period in the different decerebrate cats Appelberg (1963) has published a record showing a large reduction in the duration of the pause of the discharges from a primary ending during a twitch on dynamic fusimotor activation

Certain problems concerning the excitation and functional role of the Golgi tendon organs are also raised by the present study It was a striking observation that the tendon organs were much more responsive to active contraction than to passive extension of the muscle This is most easily explained on the basis of a difference in the distribution of the exciting force within the muscle in the two cases Particularly in a bipennant muscle like the soleus this is fairly easily visualized In such a muscle the distribution of forces must be rather different according to whether the tension is caused by passive extension or active contraction of the muscle Certainly the observation of the location of the majority of the tendon organs with a high threshold to passive extension in the proximal part of the muscle favours this explanation

But the fact remains that all the tendon organs had a low threshold to active contractions They were all excited by isometric twitches developing less than 160 g of tension and the major was excited by less than 60 g twitches 60 g is less than 10

of the force of a maximal isometric twitch in the soleus This raises the question of the possible reflex function of the signal from the Golgi tendon organs For extensor muscles this has been shown to be autogenetic inhibition (Granit 1950 Granit and Strom 1951, Laporte and Lloyd 1952 Hunt 1952) The present observation of the low and moderate threshold of the tendon organs to active contractions suggests that the tendon organ during movements continuously provides information about the degree of active contraction in the muscle Such information presumably finds an extensive application in locomotor integration Other authors have made suggestions along the same lines (Eccles and Lundberg 1959 Cruz and Hufschmidt 1962 Matthews 1964) In addition the present demonstration of the difference in threshold of the tendon organs to stretch and contraction provides an explanation for certain observations on adequate activation of spinal reflexes In such experiments it is regularly found that the alleged Golgi tendon organ effect is appreciably more pronounced during active contraction than during passive stretch of the muscles (Cooper and Creed 1927 Granit 1950 Hagbarth and Naess 1950)

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Range of Sympathetic Discharge and Reflex Vascular Adjustments in Skeletal Muscle during Hemorrhagic Hypotension

By

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Abstract

Lundgren O J Lundwall and S Mellander *Range of sympathetic discharge and reflex vascular adjustments in skeletal muscle during hemorrhagic hypotension* Acta physiol scand 1964 *62* 380—390 — Hemorrhage is associated with an increase in the sympathetic vasoconstrictor fiber discharge to skeletal muscle ranging from values below 1 imp sec up to about 7 imp sec the rate of discharge being related to the extent of bleeding The reflex increase in sympathetic activity is as a rule fairly well maintained during prolonged hemorrhage Normally this elicits a reflex constriction of resistance and capacitance vessels and an increase in the pre /post capillary resistance ratio leading to decreased capillary pressure and absorption of extravascular fluid The constriction of the capacitance vessels and the fluid absorption both tend to compensate for the reduction of intravascular fluid volume in the early phase of hemorrhage However there is a gradual decline in the reflex vascular response despite maintained vasoconstrictor fiber discharge evidently due to competitive action of locally produced dilator factors This decline is more pronounced the more severe the reduction in muscle blood flow accompanying hemorrhage The decline is more pronounced in the precapillary vessels than in postcapillary vessels Therefore in the later stages of hemorrhage the pre /postcapillary resistance ratio may decrease to such an extent that fluid escapes from the circulation by filtration leading to further derangement of cardiovascular function

It has been shown that in cat skeletal muscle the vascular reactivity to regional sympathetic vasoconstrictor nerve stimulation is decreased when the nutritional blood flow to the tissue is reduced by partial occlusion of the arterial inflow (Lewis and Mellander 1962) and when the animal is exposed to hemorrhagic hypotension (Mellander and Lewis 1963) The decline in reactivity was shown to be faster and more pronounced in precapillary than in postcapillary vessels The declining reactivity to vasoconstrictor fiber stimulation was considered to be due to a competitive dilator influence exerted by locally produced "metabolites It was suggested that the manner in which vascular reactivity was changed could aid in understanding some of the features in reversible as well as in irreversible shock.

It seems to be generally agreed that during hemorrhage there is a reflex increase in the activity of the sympathetic nervous system but there is no quantitative evaluation of the extent to which the sympathetic discharge rate is raised during various levels of hemorrhage and for how long periods of time this activity will be maintained The above mentioned investigations made it clear that due to a locally induced declining vascular reactivity an estimation of the sympathetic activity cannot directly be judged from studies of the peripheral vascular effector response This may however be done if a cross circulation technique is utilized which eliminates other influences than neural on the vascular smooth muscle cells

The aim of the present investigation was to study the reflex vascular effects in skeletal muscle during various levels of hemorrhagic hypotension The responses of the pre and postcapillary resistance vessels the precapillary sphincters and the capacitance vessels as well as changes in transcapillary fluid movement were recorded in the right calf muscle perfused from the animal itself Simultaneously the responses of the resistance vessels were followed in the left calf muscle which was cross-circulated at constant flow from a donor cat The reflex resistance effects in the cross-circulated limb during hemorrhage were compared with the effects obtained in the same limb on graded stimulation of the regional vasoconstrictor fibers performed after transfusion of the shed blood This permitted a quantitative evaluation of the changes in sympathetic discharge rate during various stages of hemorrhage

Methods

Hemorrhage experiments were performed on 18 cats In 15 of these experiments a cross circulation technique was utilized with donor cats of approximately the same weight as the experimental animal Both the recipient and donor cat were anesthetized with chloralose (40 mg/kg) and urethan (no more than 70 mg/kg)

An isolated calf muscle preparation was done on each hind limb of the recipient cat leaving the innervation intact (for details see Kjellmer 1964 Mellander Öberg and Odelram 1964) The left calf muscle was supplied with blood from a donor cat For this purpose the femoral artery of the donor cat was connected by a siliconized polyethylene tubing to the distal end of the severed left popliteal artery of the recipient This tubing was placed in a Sigmamotor pump (Model TM 11) The venous outflow of blood from the left popliteal vein was measured by a drop-recorder unit and the blood returned to the femoral vein of the donor cat Blood flow through the left calf muscle was adjusted to the flow level at rest by the Sigmamotor pump and then maintained at this level throughout the experiment Arterial perfusion pressure was monitored from a T tube distal to the Sigmamotor pump The right popliteal artery was left intact so that the right calf muscle was supplied with the recipient s own blood The venous outflow of blood from the right popliteal vein was measured with a drop-recorder unit By enclosing the right calf muscle preparation in a water filled temperature regulated plethysmograph it was possible to record phasic changes in both regional blood volume and in regional extravascular fluid volume (see Mellander 1960) In some experiments changes in regional blood volume were recorded simultaneously using a radioactive isotope monitoring technique (Åblad and Mellander 1963)

The arterial pressure in the recipient and the donor cat was recorded from indwelling catheters in the left axillary artery The blood pressure of the donor cat was kept constant throughout the experiment If pressure tended to decline small amounts of Dextran Tyrode s solution were given to the donor to minimize reflex changes in its adrenal medullary secretion

Hemorrhagic hypotension was produced in the recipient by rapid withdrawal of arterial blood into a syringe The magnitude of the hemorrhage was varied in different experiments between 10 per cent and 40 per cent of the cat s total blood volume The total blood volume of the cat amounts to 7 to 8 per cent of the body weight (Hamlin and Gregersen 1939 Mellander unpublished

To minimize red cell aggregation that may occur to a considerable extent especially in long term experiments small amounts of dextran with low molecular weight (Rheomacrodex) were given to the animals (Eliasson and Samelius Broberg 1963) This was considered important to avoid passive changes in regional resistance due to blood cell sludging which could be misinterpreted as an active constriction of the resistance vessels

In some experiments Dibenzyline was administered i a to the region studied (1—3 mg/kg) in order to block the action of the sympathetics during the hemorrhagic hypotension

Graded supramaximal electrical stimulation of the severed sympathetic chains at the level of the 4th lumbar ganglion wa performed in the recipient cat towards the end of the experiment when the shed blood was returned and the vascular functions were restored to normal The vascular responses in the cross-circulated limb obtained at known frequencies of stimulation were compared with those reflexly produced during hemorrhagic hypotension to evaluate the rate of the sympathetic discharge occurring during hemorrhage

With the technique used in the present experiments it was possible to follow continuously changes in the resistance function (pressure flow) in the capacitance function (change of blood volume) as well as net movements of fluid across the capillary walls (change of tissue volume minus change of regional blood volume) In addition by determining the capillary *filtration coefficient CFC (ml fluid filtered/m n × 00g × mm Hg pressure gradient) information* could be gained on changes in the size of the capillary surface area available for exchange (see Cobbold *et al* 1963)

Results

The first part of this chapter gives a description of the constrictor responses of the resistance vessels in a cross circulated muscle vascular bed induced by hemorrhage and by stimulation of the regional vasoconstrictor fibers These data were used for an evaluation of the discharge rate in the sympathetic vasoconstrictor fibers during hemor rhage The second part gives a detailed analysis of the pattern of response in the various consecutive vascular sections occurring during hemorrhagic hypotension in a muscle preparation perfused from the animal itself Experiments were performed at three levels of hemorrhage In one group 10 per cent of the cat s total blood volume was withdrawn (below called small bleeding) in a second 15 to 20 per cent (moderate bleeding) and in a third 30 to 40 per cent (large bleeding) Since the bulk of data was obtained during moderate bleeding the results of these experiments will be described first and comparison then made to the effects observed at the other levels of hemorrhage

During moderate hemorrhage blood flow decreased markedly in the autoperfused limb The decrease was most pronounced in the early stage when flow amounted to only about 1/4 to 1/5 of resting flow In the cross circulated limb flow was kept constant at the resting value throughout the experiment Fig 1 illustrates the reactions of the resistance vessels occurring in the cross circulated and the autoper *fused limb during moderate hemorrhage* The changes in regional resistance are expressed in per cent of the resistance prevailing in the control period In the cross circulated limb resistance increased initially to about 120 to 140 per cent above con trol After this transient peak resistance stabilized at a level of about 100 per cent increase and the resistance response showed then only a slight gradual decline during the remaining period of bleeding When the shed blood was returned to the animal the resistance rapidly returned to or slightly below the control level The resistance increase during hemorrhage was shown to be due to an increase in the sympathetic discharge since the response was completely abolished by sectioning the regional sympathetic vasoconstrictor fibers or by the administration of Dibenzyline

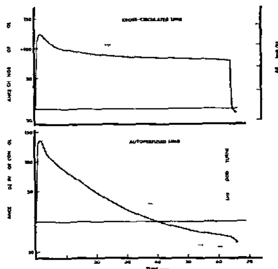


Fig 1 Effect on the resistance vessels to moderate bleeding in a cross-circulated and in an autoperfused calf muscle preparation Responses expressed as percentage of the control value at rest Shaded areas indicate range of 8 experiments Blood flow decreased during hemorrhage to about 1/4 of control flow in the autoperfused limb but was kept at normal level in the cross circulated limb In both limbs hemorrhage is associated with an initial pronounced reflex increase in resistance After this the resistance response of the autoperfused limb rapidly declines while it is well maintained in the cross circulated limb The change in the discharge rate in the sympathetic vasoconstrictor fibers to skeletal muscles is indicated along the right ordinate of the upper diagram

In an attempt to evaluate quantitatively the change in the rate of discharge in the sympathetic nervous system occurring during hemorrhagic hypotension the reflex effects in the resistance vessels above-described were compared with the resistance responses obtained by graded supramaximal stimulation of the lumbar vasoconstrictor fibers to the same limb The results of such experiments expressed as means are indicated by the ordinate on the right hand side of Fig 1 It can be seen that the discharge at rest amounted to approximately 0 5 imp/sec During the initial phase of the moderate bleeding the discharge rose to 3 to 4 imp sec and at the more steady phase it amounted to 2 to 3 imp/sec slowly declining with time Evidence was presented that the discharge of the sympathetics could be maintained constantly at about 3 imp/sec for a considerable length of time (at least for 2 hours) if the decrease of the intravascular volume was kept at a constant level during the whole period of hemorrhage This implied as will be described below that the magnitude of changes in intravascular volume produced by the response of the capacitance vessels in terms of redistribution of their blood depot and by transcapillary movement of fluid was known and that such alterations were compensated for continuously

In the autoperfused limb which was exposed to the spontaneous changes in flow that occur during hemorrhage as a rule a reduction to 1/4 to 1/5 of normal flow there was an initial peak resistance response of about the same magnitude as in the cross-circulated one (Fig 1 lower panel) Fairly soon however the resistance response declined and resistance reached the control level after 20 to 60 min and later on the values obtained were even below control After transfusion of the shed blood there was a slight further dilatation of the resistance vessels This pattern of declining resistance response during bleeding is similar to that found during hemorrhagic hypotension when the regional sympathetics were stimulated directly (Mellander and Lewis

To minimize red cell aggregation that may occur to a considerable extent especially in long term experiments small amounts of dextran with low molecular weight (Rheomacrodex) were given to the animals ([illegible] 1963). This was considered important to avoid passive changes in regional resistance due to blood cell sludging which could be misinterpreted as an active constriction of the resistance vessel.

In some experiments Dibenzyline was administered close to the region studied (1—3 mg/kg) in order to block the action of the sympathetics during the hemorrhagic hypotension.

Graded supramaximal electrical stimulation of the severed sympathetic chain at the level of the 4th lumbar ganglion was performed in the recipient cat towards the end of the experiment when the shed blood was returned and the vascular functions were restored to normal. The vascular responses in the cross-circulated limb obtained at known frequencies of stimulation were compared with those reflexly produced during hemorrhagic hypotension to evaluate the rate of the sympathetic discharge occurring during hemorrhage.

With the technique used in the present experiments it was possible to follow continuously changes in the resistance function (pressure/flow), in the capacitance function (change of blood volume) as well as net movements of fluid across the capillary walls (change of tissue volume minus change of regional blood volume). In addition, by determining the capillary filtration coefficient CFC (ml fluid filtered/min/100 g/mm Hg pressure gradient) information could be gained on changes in the size of the capillary surface area available for exchange (see Cobbold *et al.* 1963).

Results

The first part of this chapter gives a description of the constrictor responses of the resistance vessels in a cross-circulated muscle vascular bed induced by hemorrhage and by stimulation of the regional vasoconstrictor fibers. These data were used for an evaluation of the discharge rate in the sympathetic vasoconstrictor fibers during hemorrhage. The second part gives a detailed analysis of the pattern of response in the various "consecutive" vascular sections occurring during hemorrhagic hypotension in a muscle preparation perfused from the animal itself. Experiments were performed at three levels of hemorrhage. In one group 10 per cent of the cat's total blood volume was withdrawn (below called "small" bleeding), in a second 15 to 20 per cent ("moderate" bleeding) and in a third 30 to 40 per cent ("large" bleeding). Since the bulk of data was obtained during "moderate" bleeding the results of these experiments will be described first and comparison then made to the effects observed at the other levels of hemorrhage.

During "moderate" hemorrhage blood flow decreased markedly in the autoperfused limb. The decrease was most pronounced in the early stage when flow amounted to only about 1/4 to 1/5 of "resting" flow. In the cross-circulated limb flow was kept constant at the "resting" value throughout the experiment. Fig. 1 illustrates the reactions of the resistance vessels occurring in the cross-circulated and the autoperfused limb during "moderate" hemorrhage. The changes in regional resistance are expressed in per cent of the resistance prevailing in the control period. In the cross-circulated limb resistance increased initially to about 120 to 140 per cent above control. After this transient peak, resistance stabilized at a level of about 100 per cent increase and the resistance response showed then only a slight gradual decline during the remaining period of bleeding. When the shed blood was returned to the animal the resistance rapidly returned to or slightly below the control level. The resistance increase during hemorrhage was shown to be due to an increase in the sympathetic discharge since the response was completely abolished by sectioning the regional sympathetic vasoconstrictor fibers or by the administration of Dibenzyline.

In Fig 2 (lower panel) the changes in CFC during the hemorrhage are indicated In the control period CFC amounted to 0.015 which is within the normal range for resting cat skeletal muscle A few minutes after the commencement of the bleeding CFC amounted to 0.011, but soon increased again After 6 to 7 min it reached 0.025 and did not change significantly during the remaining period of bleeding This suggests that in the earliest phase there is some nervous constrictor influence on the precapillary sphincters reducing the perfused capillary surface area (cf Folkow and Mellander 1960 Cobbold *et al* 1963) Very soon however this constriction is reversed to a dilation above the control level to judge from the rise in CFC presumably due to the action of accumulated metabolites (Lewis and Mellander 1962)

The lower curve of Fig 2 illustrates the rate and direction of net fluid movement across the capillary walls during hemorrhage The bleeding was associated with an absorption of extravascular fluid into the intravascular compartment which initially amounted to almost 0.09 ml/min × 100 g muscle With time the rate of absorption decreased and after about 25 min of hypotension and relative ischaemia capillary exchange was reversed to a filtration of fluid from the circulatory system into the extra vascular space at a rate which approached 0.04 ml/min × 100 g muscle This shows that fairly large volumes of fluid can be absorbed from the tissues and added to the venous return during the early stages of hemorrhage Conversely the circulating plasma volume seems to diminish by outward filtration into the tissues in the later stages of hemorrhage

Absorption of extravascular fluid into the circulatory system is part of the normal response in skeletal muscle to activation of the vasoconstrictor fibers (Mellander 1960) This effect is due to a relatively more pronounced increase in the precapillary than in the postcapillary resistance producing a decrease of mean hydrostatic capillary pressure The present investigation indicates in agreement with Öberg s observations (1964) that this effect can also be produced by reflex activation during hemorrhage This response is however reversed to an outward filtration in later stages of hemorrhage presumably due to a decrease below control of the precapillary to postcapillary resistance ratio leading to an increase of mean capillary pressure above control It should be noted that the outward filtration appears at the stage when the resistance response (mainly precapillary) is virtually abolished while the capacitance response (mainly postcapillary) is still well maintained The experimental evidence suggests that the phenomenon of outward filtration is the result of a changed reactivity within the pre and postcapillary resistance vessels to vasomotor fiber influence (cf Lewis and Mellander 1962 Mellander and Lewis 1963) Further support for this interpretation was provided by the following findings If the sympathetics were blocked by Dibenzyline during the phase of outward filtration further loss of fluid from the circulatory system was prevented Furthermore if during the phase of outward filtration the recipient animal was exposed to an additional bleeding which increased the sympathetic discharge (as judged by a considerably increased peripheral resistance in the cross-circulated limb) there was simultaneously in the autoperfused limb at least temporarily a slight increase in total resistance and a disappearance of the outward filtration phenomenon It therefore seems as if an increase in the sympathetic discharge at this stage still can affect the precapillary resistance vessels so that the pre to p resistance ratio returns towards normal Conversely Dibenzyline seemed to restore the pre to postcapillary resistance ratio towards normal

TABLE I

Magnitude of hemorrhage	10 per cent			15–20 per cent			30–40 per cent		
Time after onset of hemorrhage	3 min	15 min	40 min	3 min	15 min	40 min	3 min	15 min	40 min
Symp discharge imp/sec	[illegible]–3	[illegible]	[illegible]1 [illegible]	3–4	2–3	2–3	3–7	3–5	3–5
Resistance response % of maximum	100	60	0	100	50	0	100	20	below control
Capacitance response % of maximum	100	95	50	100	9	80	100	40	60
Fluid amount absorbed (ml/min/100 g)	0.015	0.045	0.010	0.035	0.01	–	0.100	0.0[illegible]5	–
Fluid amount filtered ml/min/100 g	–					0.010	–	–	0.050
Change of Pc mm Hg decrease increase	4	[illegible]	0.5	8			−11	−1	−2

blocking the remaining sympathetic influence, namely on the postcapillary resistance vessels and the capacitance vessels.

The extent to which mean capillary hydrostatic pressure was changed during hemorrhage can be calculated when both the rate of net capillary fluid exchange and CFC are known. As indicated in Fig. 2, mean capillary pressure (Pc) fell to values considerably below control in the earlier stages of a "moderate" bleeding. Conversely it rose to values above control in the later stages of hemorrhage.

When the shed blood was returned to the recipient animal there was, as a rule, a brief period during which the smooth muscles of the different vascular sections seemed to be relaxed beyond the control level. Within 10 to 20 min there was, in almost all experiments, a complete recovery of the vascular functions.

In the present investigation the reflex response patterns were also followed when the recipient cat was bled 10 per cent ("small" bleeding) and 30 to 40 per cent of its blood volume ("large" bleeding). During the "small" bleeding nutritional blood flow of the calf muscle was reduced initially to about 1/2 of control and to about 1/5 to 1/8 during the "large" bleeding. Table I summarizes the results obtained expressed as mean values of 5 experiments in each group. It can be seen that the general patterns of response were similar to that during "moderate" bleeding. The differences between the three series of experiments are mainly quantitative. In some respects, however, the patterns differed from that described above. During the "small" bleeding the resistance response in the autoperfused limb hardly ever declined to or fell below the resistance in the control period, and the phenomenon of extravascular fluid absorption was never reversed to an outward filtration. Further, at this level of bleeding the compensatory mechanisms of the organism often seemed to be sufficient to permit a

spontaneous return of the vascular functions to normal sometimes in less than an hour When very large amounts of blood were withdrawn and the arterial blood pressure fell to levels below 30 to 40 mm Hg a gradual but eventually pronounced fall in the resistance response of the cross-circulated limb was sometimes observed If however in such situations small amounts of blood were given back to the recipient so that blood pressure rose above 50 mm Hg there was often an abrupt and sustained increase of resistance These data suggest a temporary insufficiency of central sympathetic structures as will be discussed below In some of the experiments with large bleeding there was not a complete recovery of the vascular functions upon transfusion of the shed blood

Discussion

The present investigation has shown that the discharge rate in the sympathetic vasoconstrictor fiber system to skeletal muscle is increased from a "resting" value below 1 imp/sec to about 6 to 7 imp with large bleedings (30 to 40 per cent of the cat's blood volume) The discharge rate is roughly proportional to the extent of the hemorrhage at small bleeding (10 per cent) 2 to 3 imp/sec at moderate bleeding (15 to 20 per cent) 3 to 5 imp/sec These values are well within the physiological discharge range (up to about 8 imp/sec) (Folkow 1952) Since an exact evaluation of the sympathetic discharge to a peripheral vascular section can hardly be done with a direct electrophysiological technique due to the great difficulty of isolating a single and at the same time representative postganglionic nerve fiber for spike potential recording the indirect method of correlating the reflex effector response with that obtained on direct supramaximal stimulation of the regional vasoconstrictor fibers was used It may not be taken for granted that *in vivo* there is always an engagement of all sympathetic fibers *simultaneously but there are reasons to believe that this method will give* approximately correct values (see Folkow 1952)

The reflex increase in the activity of the sympathetic nervous sytem was characterized by an initial transient peak during hemorrhage After this there was a slight gradual decline in the discharge rate The experimental evidence suggests that two different mechanisms may explain at least in part this gradual decline First there is an absorption of extravascular fluid which tends to restore the intravascular volume This absorption of fluid was sometimes so large that an almost complete replacement of the shed blood volume occurred Such a sufficient compensation was especially noted during small bleedings and then the discharge in the sympathetic system returned towards the resting level Second in some exceptional cases there was an indication of a central insufficiency of the sympathetic nervous system This was noted in a few experiments with large bleeding when arterial blood pressure fell below 30 to 40 mm Hg (cf Rothe Schwendenmann and Selkurt 1963) It seemed that in such situations the nutritional blood flow to the central nervous system was inadequate for maintaining an undisturbed activity It was fairly easy to differentiate between these two situations of declining discharge *since transfusion of small amounts of blood* to the animal in the first case produced a further derease of the peripheral resistance in the cross-circulated limb while in the latter case transfusion led to an increased resistance

The present study indicates that in most cases hemorrhage is associated with a fairly pronounced and sustained increase in the activity of the sympathetic vaso-

constrictor fiber system. This influence results, however, in very different constrictor effects within the various consecutive sections of the autoperfused skeletal muscle vascular bed. There is a general tendency for vascular reactivity to decline with time, but the decline is much more pronounced in the precapillary section (precapillary resistance vessels and precapillary sphincters) than in the postcapillary section (post-*capillary resistance vessels and main capacitance vessels).* This pattern of changing reactivity is very similar to that seen during direct stimulation of the regional sympathetic vasoconstrictor fibers to skeletal muscle when its nutritional blood flow was mechanically reduced (Lewis and Mellander 1962) or when the animal was exposed to hemorrhagic hypotension (Mellander and Lewis 1963). These two previous studies made it clear that the declining reactivity was due mainly to a competitive effect upon the constrictor fiber influence exerted by dilator "metabolites" accumulating in the tissue when nutritional blood flow was reduced. Since a substantially reduced nutritional blood flow to skeletal muscle is a dominant feature also in the present series of experiments, it is reasonable to believe that a similar change in local chemical environment is the main factor responsible for the declining vascular reactivity. The present preparation was closer to "normal" than those used in the above-cited studies, since in this study the adrenal medullae, the sympathetic cholinergic vasodilator fiber system and the gastrointestinal tract were left intact. It might be argued that possible release of medullary catechol amines (adrenaline), engagement of the sympathetic vasodilator system or possible absorption of deleterious materials from the gastrointestinal tract could be factors that would contribute to the declining vascular reactivity. Since, however, a very similar general pattern of response was obtained in all three studies, even quantitatively, it may be concluded that such extrinsic factors did not modify to any greater extent the pattern of vascular reactivity seen in the skeletal muscles, at least not in the short term experiment.

The present study indicates, in agreement with Öberg (1963, 1964), that the adequate compensatory mechanism for hemorrhage, a restoration of the circulating intravascular volume, is in part accomplished by the reflex adjustments in the muscle vascular bed. By a sustained constriction of the capacitance vessels, their blood "depot" is made available for the central circulation and by a reflex increase of the pre- to postcapillary resistance ratio extravascular fluid is absorbed into the circulatory system. If the quantitative data on the capacitance response and on the transcapillary fluid absorption found in the calf muscle are representative for all cat skeletal muscle, it can be calculated that during "moderate" hemorrhage in a medium-sized cat a total of about 5 to 10 ml of blood would be mobilized by the reflex constriction of the capacitance section. Further, during the initial 20 min period some 10 to 15 ml of fluid would be absorbed *from the extravascular space of skeletal muscle. This gain corresponds* to about half of the blood volume shed. During a "small" bleeding these compensatory effects are slightly less pronounced but may, on the other hand, be maintained for a longer period of time. Therefore, there may often be an almost complete restoration of the intravascular volume by these means and, with this, as mentioned above, also a return of the vascular reactivity and the sympathetic discharge level towards normal. For a full discussion of these problems the reader is referred to the recent study by Öberg (1963, 1964).

The reflex constrictor responses of the precapillary resistance vessels and the precapillary sphincters during the hemorrhage are tempered to a great extent by locally produced "metabolites". This declining reactivity may be advantageous for the tissue

since its nutritional blood supply will not be too seriously interfered with provided cardiac performance is adequate Further by opening of the precapillary sphincters the available blood will be spread out over a greater capillary network favouring capillary filtration and diffusion exchange and tissue nutrition One aspect of this is illustrated in Fig 2 By an increase in CFC during hemorrhage the rate of fluid absorption could be maintained at a fairly constant level for considerable length of time despite the fact that the reduction in mean hydrostatic capillary pressure was especially pronounced only during the first few minutes of hemorrhage

In later stages of moderate and large bleedings it appears that the reflex engagement of the sympathetics with regard to skeletal muscle circulation may act in a decompensatory manner The maintenance of the postcapillary resistance response in the face of a markedly declined precapillary resistance response will so change the pre to postcapillary resistance ratio that fluid is filtered from the circulation to the extravascular space In this way 5 to 10 ml of fluid may be lost from the circulation in a 20 min period in a medium sized cat If such a drainage of fluid continues for a considerable length of time it may so seriously interfere with the circulatory homeostasis that the organism may not survive Blockade of the sympathetics by Dibenzyline was shown to prevent further fluid loss in this stage It is possible that this finding can help to explain the beneficial effect of sympathicolytics in shock (see Nickerson 1963) Such treatment may prevent further derangement of the peripheral circulation but does not seem to be able to contribute actively to recovery More adequate treatment is of course accomplished by transfusion and the present study demonstrates that fairly prompt restitution of vascular reactivity towards normal may then be obtained even in the stage of outward filtration or failure in the central vasomotor system This implies that the decompensatory effects of the reflex engagement will be reversed upon transfusion so that the pattern of compensatory vascular effects is again called into play aiding a restitution towards normal During prolonged and severe hemorrhage there may not be complete recovery of the vascular functions with transfusion and in such situations an irreversible shock might develop Obviously there are still other factors that might be responsible for the irreversibility of shock such as myocardial insufficiency etc

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The Effect of Parathyroid Hormone and Vitamin D on Serum Calcium in Rats

By

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Abstract

Toverud S U *The effect of parathyroid hormone and vitamin D on serum calcium in rats* Acta physiol scand. 1964 62 391—406 — Conflicting evid nce exists rega ding the hypothesis that ta n n D is ess ntial for the calc um mob lizing action of p rathyroid hormone A re investigation was undertaken by feeding weanling vitam n D-deficient rats a vitamin D free d et containing 0 7 % calc um and 0 6 phosphorus (diet D) for 3 eeks At day 1 when th mean serum calc um level was 7 1 mg/100 ml the d t was replaced by 10 glucose in saline Diffe ent groups received e ther A) 100 I U vitam n D on day 2 B) njections of 200 and 100 units para thyro d extract (PTE) on days 4 and 5 respectively C) a comb nation of treatments A and B or D) saline injections The serum calcium levels determined 6 hours after the last PTE injec tion permitted calculation of mean ind vidual increase expressed n mg/100 ml A) + 1 04 B) + 0 86 C) + 2 58 D) − 0 57

Continuous feeding w th d et D or one containing less p ote n resulted also in a significant response to PTE Parathyroidectomy but n t ham operation resulted in a substantial fall in serum calcium in rats fed diet D

The conclus ons are 1) The calc um mobilizing action of p rathyroid hormone in the rat is either independent of or requires only minute amounts of itamin D 2) At the one dos level invest gated no e idence for inte act on between the effects of vitamin D and parath roid extract was found wh n intestinal calc um bsorption was minimal

Vitamin D is now regarded as one of the two major factors which maintain a normal plasma calcium level (McLean 1960) the other factor being the classic parathyroid hormone This concept is partly based on experiments in vitamin D-deficient rats show ing that vitamin D can bring about an increase in the serum calcium level which is partly independent of the effect of the vitamin on intestinal calcium absorption (Carls son 1952 Nicolaysen and Eeg Larsen 1956) Recently it has been claimed that vitamin D has the additional role in calcium homeostasis of permitting the parathyroid hormone to induce its action upon the bone and possibly also other target organs (Rasmussen and Reifenstein 1962) This postulate is partly founded upon experiments by Harrison Harrison and Park (1958) showing that injections of parathyroid extract into vitamin D-deficient young rats did not raise the serum calcium level unless the rats were pre treated with vitamin D Similar results have been obtained by Marnay and Raoul (1959)

TABLE I Composition of vitamin D free diets[1]

	High protein (%)	*Low* protein[2] (%)	Stock diet[3] (%)
Dextrose	—	23.6	—
Cornstarch	—	42.5	—
Ground whole wheat rye oats and corn	—	—	60
Wheat germ		—	5
Wheat flour (70% extraction)	59.8	—	—
Casein acid precipitated	20	17	—
Egg albumin crystalline	5	—	—
Dried skimmed milk	—	—	24
Arachis oil	10	7.5	5
Brewer's yeast	—	4.7	5
B-vitamin mixture	0.1	—	—
Salt mixture		4.7	—
Salt mixture (Ca and P free)[4]	2.0		—
Choline chloride	0.05		—
Calcium carbonate	1.75	—	5
Potassium diphosphate	1.32	—	—
Sodium chloride	—	—	5
Protein	31	18	16
Calcium	0.70 – 0.73	0.61	0.57
Phosphorus	[6]0.54 – 0.59	0.67	0.54

[1] Containing no known sources of vitamin D
[2] As described by Harrison et al (1958)
[3] Supplements are described in the text
[4] As described by Coran (1960a)
[5] As described by Wesson (1932)
[6] Range of 6 batches (6 samples of each)

and Marnay (1961) Most recently Rasmussen et al (1963) reported results that were confirmatory with regard to low and moderate doses of parathyroid extract However treatment with massive doses of the purified hormone resulted in a response also in vitamin D-deficient rats Other workers have demonstrated that vitamin D deficiency does not prevent the calcium mobilizing action of moderate doses of parathyroid extract in adult rats (Toverud 1963) mice (Nichols Schartum and Vaes 1963) and hens (Hertelendy and Taylor 1960)

The relationship between vitamin D and parathyroid hormone has also been studied on the level of cell mitochondria from rats It has been demonstrated that parathyroid hormone stimulates a vitamin D-dependent release of calcium from kidney mitochondria (DeLuca Engstrom and Rasmussen 1962) and a vitamin D independent uptake of inorganic phosphate by liver mitochondria (Sallis DeLuca and Rasmussen 1963)

Because of the discrepancy in results from experiments *in vivo* and because of the importance of this type of information for the understanding of the mechanism of action

of vitamin D and parathyroid hormone it was desirable to re investigate the separate and combined effects of the two agents on the serum calcium level in mature and young vitamin D-deficient rats In addition young rats suffering from vitamin D deficiency were subjected to parathyroidectomy or a sham operation

A preliminary account has been given at the 6th International Congress of Nutrition Edinburgh Aug 9—15 1963 (Toverud 1964)

Materials and methods

Rats

All rats came from the same colony of interbred strains of hooded and albino rats which has been maintained at this institute for over 30 years without additions from other colonies. Diets containing minimal amounts of vitamin D have been given to the stock rats for the last 20 years The rats were weaned when approximately 24 days old After a pre treatment period of 20 days or longer rats of both sexes were divided into treatment groups by formal randomization. In the experiment with adult rats only females were used and the pre treatment period started at the age of 6 months

Diets

The composition of the diets is given in Table I The mothers of the experimental rats were fed during pregnancy and lactation the stock diet supplemented with whole milk ad lib tum and weekly rations of bread boiled meat and carrots or spinach In general, skimmed milk was substituted for whole milk during the last half of the lactation period. From time of weaning and throughout the experimental period the rats were fed one of the two vitamin D-free purified diets except in one experiment (37) in which this diet was replaced by a calcium free regime consisting of a solution of 10 per cent glucose and 0 9 per cent NaCl in de ionized water (later referred to as glucose in saline diet) during the period of administration of vitamin D and parathyroid extract. All rats received supplements of 150 I U vitamin A-acetate every two days except the rats in exp 37 while they received the glucose in saline diet

Criteria for vitamin D deficiency

The pregnant and lactating mothers of the experimental rats were fed the same diet as mothers of rats used regularly in this laboratory for vitamin D assay (2 point factorial dose radiographic curative assay as described by Bliss and Gyorgy 1951) After consuming a rachitogenic (high calcium, low phosphorus) diet for 10—12 days from the time of weaning these rats develop severe rickets as evidenced by lack of roentgenologically visible epiphyseal calcification of the proximal end of the tibia A substantial healing response is frequently obtained with as little as 0 4 units of vitamin D daily for 12 days and regularly with 0 8 units indicating that the rats contain only traces of vitamin D prior to assay Since the assay rats and the present rats had identical maternal and dietary backgrounds until time of weaning since the subsequent diets for the present rats contained no known source of vitamin D and since the two groups were equally exposed to daylight it was assumed that the present rats had about the same degree of vitamin D deficiency as the rachitic assay rats

Parathyroidectomy

Parathyroidectomy was carried out by means of hot wire cautery as described in detail by Hirsch Gauthier and Munson (1963)

Bleeding

1—1 5 ml samples of blood were taken from the tail while the rats were lightly anesthetized with ether In general blood samples were taken immediately before and after parathyroidectomy or the periods of treatment with parathyroid extract or vitamin D respectively The time of the latter sample will be referred to as the second bleeding

Parathyroid extract and vitamin D

The parathyroid extract was assayed by El Lilly Co to contain 375 units per ml. The extract was stored at 7 C for 1 5 years prior to use and may not have retained its full potency When parathyroid extract was given the animals received by subcutaneous injection 100 and 100 units 4 and 6—8 hours, respectively before the second bleeding assuming full potency of the

TABLE II Precision of calcium determinations expressed as the standard deviation of a single determination (S D Eq 1)

Number of samples	Serum calcium range (mg 100 ml	S D (mg/100 ml
7	3 5 – 4 1	0 09
20	4 3 – 6 7	0 0[illegible]
25	[illegible] 10 4	0 0[illegible]

extract Comparable injections into control rats contained an equal volume of physiological saline solution. In the experiment with adult rats 0 [illegible] ml parathyroid extract (780 units was injected

Vitamin D (Dchysfral D — 2000 Philips — Duphar) was administered in arachis oil by dropper pipette 3 days before bleeding. This and similar preparations are routinely standardized in this institute by the above mentioned … point bioassay

Calcium analyses

Calcium analyses were carried out in duplicate on 0 [illegible] ml aliquots of serum from freshly collected blood according to the method of Munson et al. 19[illegible] with the following modifications a) Into the cuvette were pipetted 6 ml deionized water and 0.2 ml of an aqueous solution (1 mg ml) of ammonium purpurate in addition to serum 5 drops of 9 N NaCl and 1 drop of octyl alcohol b With the cuvette placed in a Coleman Junior Spectrophotometer 0 1 ml increments of a solution of EDTA (0 18 g 1 were added

The accuracy of the method was tested in a recovery experiment The addition of 10 μg calcium to each of 13 serum samples containing from 13 8 to [illegible] 0 1 μg calcium resulted in a mean recovery of 10 1 μg The standard deviation of this series was 0 4 μg corresponding to a concentration of 0.2 mg 100 ml In Table II the precision of the method is again indicated by the standard deviation based on the differences between the 2 determinations of each sample (Eq 1

It is of interest to note that Wilk and King (19[illegible]3 in comparing several calcium micro-procedures found only one procedure that of Munson et al (19[illegible] to be precise[1] to 0.2 mg 100 ml calcium.

Phosphorus analyses

Phosphorus in the diets was determined by the method of Fiske and SubbaRow (19[illegible]) modified so as to ensure complete hydrolysis of any pyrophosphate present after digestion of the diet with nitric and perchloric acids

Statistical treatment

1 Precision of calcium determination The precision was defined as the standard deviation S D) of a single determination calculated as follows

$$S\,D = \sqrt{\frac{1}{[illegible]}\sum_{1}\sum_{1} x_j \quad x} \qquad \text{Eq 1)}$$

where subscript *i* refers to individual serum samples subscript *j* refers to the first or second determination of the *i*th sample and refers to the number of samples in each series 7 20 or 25 *2 Test for additivity of separate effects of the treatments in experiment 34* Analysis of variance 2 way classification with interaction *Assumption* A correction term may possibly be added to the sum of the separate effects. *Null-hypothesis* The correction term (interaction between the main effects) is equal to zero Since the number of observations differed from one cell to the other the formulas for the case of unequal numbers of observations were used for calculating the F value (variance ratio) as described by Scheffe (1959)

[1] Precision was defined as the maximum deviation between the values for each sample when multiple determinations were carried out on several 0 2 ml serum samples (Wilk 1963)

TABLE III Effect of vitamin D and/or parathyro d extract on the serum calcium of para thyroidectomized vitamin D deficient adult female rats

Mean ± standard error of 7 rats in each group The 24 month-old rats had been fed for 18 months the vitamin D free stock diet with weekly supplements of carrots or spinach The doses of itamin D (D) and parathyro d extract (PTE) were 250 I U and 270 units respecti ely Range of body weight 185—256 g

D ys after PTX	Serum calcium (mg/100 ml) Group I		Group II		Group III	
	Level	Rise	Level	Rise	Level	Rise
6	4 46±0 23	—	5 20±0 26	—	4 62±0 18	—
10	—	—	D	D	D	D
12	PTE	PTE	—	—	PTE	PTE
13	6 67	2 ?0 ±0 23	7 16	1 96±0 31	8 88	4 ?5±0 24
20	5 52	—	6 33	—	6 41	—
20	PTE	PTE	PTE	PTE	—	—
21	6 80	1 28±0 17	8 15	1 8?±0 19	—	—
25	5 15	—	6 52	—	—	—

Analyzed in single 0 5 ml samples of serum according to the method of Munson et al (1955) without the noted modifications

Parathyroidectomy

Individual rise above the previous level

* 6 rats

3 *Test for the eff ct of sex on the response to pa athyroid extract* A test for addit vity of separate effects of parathyroid extract and sex was based on results from rats eating 2 different d ets (expts 37 and 41) Analysis of variance 3 way classification with nteractions the 3 factors being parathyroid ext act sex and diet *Assumption* A correction term may poss bly be added to the sum of the ? first separate effects *Null hypothesis* The correction term (nteraction be tween the 2 first separate effects) is equal to zero The usual analysis of ariance test for this purpose was used fo calculating the F value (variance ratio) (Scheffe 1959)

4 *t test* After the analysis of variance in the form of a 2 way class fication as carried out (exp 37) the standard dev ation within cells was used as the basis fo a t test (51 degrees of freedom) with the purpose of testing the s gnificance of the mean indi dual change in seru n calc um n cells (groups) I and II

$$t = \bar{x}/S\,D\,/\,n \qquad \text{(Eq 7)}$$

where n is the number of observations n the ell and S D (standard dev ation within cells) is 0 68 (N = 55 d.f = 51)

Results

Adult rats

In the first experiment 24 month-old female rats were parathyroidectomized after ha ing consumed the vitamin D-free stock diet for 18 months Six days after the opera tion the serum calcium level was uniformly low thus indicating complete removal of

TABLE IV Plan of the experiment on the effect of parathyroid extract (PTE) and/or vitamin D on the serum calcium of vitamin D-deficient young rats

On the day of the first bleeding six week old rats having been fed the high protein diet for 20 days were given a solution of 10 per cent glucose and 0 9 per cent NaCl in de ionized water instead of solid food and tap water

Time before second bleeding	Group I Control	Group II PTE	Group III Vit D	Group IV Vit D + PTE
4 days	First bleeding	First bleeding	First bleeding	First bleeding
3 days	—		Vit D	Vit D
24 hrs	0 9 NaCl	PTE 200 u		PTE 200 u
6—8 hrs	0 9 NaCl	PTE 100 u		PTE 100 u
0 hrs	Second bleeding	Second bleeding	Second bleeding	Second bleeding

* 100 I U   0 [illegible] ml   0 25 ml

the glands (see Table III) On the 10th day rats in groups II and III received 250 I U vitamin D and 2 days later the rats in groups I and III received an injection of approximately [illegible]20 units parathyroid extract Blood samples taken next day showed that there was a *mean rise in the serum calcium of approximately 2 mg/100 ml in groups I and II* and a rise twice as high in group III A subsequent return towards the base (6th day) level was indicated by the 20th day values These results strongly suggest that vitamin D-deficient parathyroidectomized rats can respond to treatment with vitamin D or parathyroid extract or both by a measurable rise in the serum calcium level However the exact magnitude of the rise cannot be equated with response to treatment since results from an untreated control group are lacking The increase in serum calcium in groups I and II one day after another injection of parathyroid extract and the return to the base (20th day) level within 5 days provides further evidence for the conclusion that vitamin D deficiency need not prevent parathyroid extract from raising the serum calcium level in adult female rats

Response to parathyroid extract and or vitamin D in young rats

In order to compare properly the effect of parathyroid extract obtained in the presence of vitamin D with that obtained in the absence of the vitamin it was necessary to eliminate or at least minimize the powerful effect of vitamin D on intestinal calcium absorption by feeding all rats a calcium free diet Increased absorption could lead to increased deposition of bone salt available for mobilization According to Munson Hirsch and Tashjian (1963) a distinction between the calcium mobilizing and the other effects of vitamin D is indicated

The plan for exp 37 is outlined in Table IV Six week old rats which had been fed the high protein diet for approximately 3 weeks were given the glucose in saline diet at the time of the first bleeding Three days before the second bleeding the rats in groups III and IV received 100 I U vitamin D 24 hours before the second bleeding

TABLE V Effect of parathyroid extract (PTE) and/or vitamin D on the serum calcium of vitamin D deficient young rats (experiment 37)

The treatment plan is given in Table IV The mean and standard error (S E) of serum calcium is given as mg/100 ml

		Group I Control	Group II PTE	Group III Vit D	Group IV Vit D + PTE
First bleeding	Mean	7 42	6 89	7 41	6 83
	S E	± 0 18	± 0 26	± 0 26	± 0 21
	No	16	11	15	13
Second bleeding	Mean	6 85	7 87	8 45	9 5[illegible]
	S E	± 0 20	± 0 21	± 0 12	± 0 17
	No	16	15	15	16
Individual increase	Mean	− 0 57	+ 0 86	+ 1 04	+ 2 58
	S E	± 0 13	± 0 25	± 0 17	± 0 15
	No	16	11	15	13

Number of observations

the rats in groups II and IV received injections of 200 units of parathyroid extract The same rats received another injection of 100 units of the extract 6—8 hours before the second bleeding The rats in group I received control injections of saline on the same two occasions

From Table V it can be seen that the initial serum calcium levels (first bleeding) were clearly below normal thus showing that vitamin D deficiency had been achieved Both the mean final calcium level (second bleeding) and the mean individual increase for each group from first to second bleeding illustrate the effects of the 4 treatments but only the individual increase will be used in the following discussion A statistically significant ($P < 0\ 01$) decrease was observed for the control group Undoubtedly this is because there was a negligible amount of calcium in the diet.

In group II the parathyroid extract treatment had resulted in an increase in serum calcium of almost 0 9 mg/100 ml significant by t test at $P < 0\ 001$ This result agrees with the observations in the adult rat described above but not with those of Harrison et al (1955) The response to vitamin D in group III was approximately 1 mg/100 ml which means that a calcium free regime does not prevent a rise in serum calcium after vitamin D administration

The combined vitamin D-parathyroid extract treatment in group IV resulted as expected in a much greater rise in serum calcium than did the other treatments The serum calcium response in this group is very nearly equal to the algebraic sum of the corresponding values for the three first groups thus suggesting that the effects of vitamin D and parathyroid extract given together are additive In order to test the hypothesis of additivity an analysis of variance was carried out in the form of a two-way classification with interaction The design of the test is indicated in Table VI where the mean individual increase in serum calcium for each group (Table V) has been written in its

TABLE VI Design of test for additivity of main effects of parathyroid extract (PTE) and vitamin D (D) (experiment 37)

$\bar{x}$ refers to the mean individual increase in serum calcium (Table V)

	No PTE	PTE
No D	Group I $\bar{x} = -0.57$	Group II $\bar{x} = -0.86$
D	Group III $\bar{x} = +1.04$	Group IV $\bar{x} = +2.58$

TABLE VII Body weight before and weight loss after feeding rats a 10 per cent glucose in saline solution (experiment 37)

The rats were re-weighed after 3 or 4 days feeding The figures are mean standard error (S E) and number of observations (No)

Sex		Body weight (g)	Period (days)	Weight loss (g) Group I	Group II	Group III	Group IV
♂ + ♀	Mean	—	3	14.7	13.5	—	—
♂ + ♀	Mean	—	4	19.2	14.5	13.3	15.1
	S E	—		± 1.0	± 0.9	± 1.1	± 0.9
♂	Mean	105.9	4	21.5	14.5	14.1	16.3
	No	28		8	6	7	7
	S E	± 1.9					
	Mean	89.4	4	16.6	14.4	12.6	13.7
	No	26		7	5	8	6
	S E	± 1.5					

appropriate cell The null hypothesis to be tested was that the interaction between the separate effects of vitamin D and parathyroid extract is equal to zero (absence of interaction is equivalent to additivity) The standard deviation (S D) within cells (N = 55 d f = 51) is 0 68 mg/100 ml and the F value (variance ratio) is 0 09 with 1 and 51 degrees of freedom Since the critical F value for the 5 per cent significance level is 4 03 and thus far greater one cannot reject the null hypothesis of the interaction being equal to zero It must therefore be concluded that the present data give no evidence against the hypothesis that the effects of vitamin D and parathyroid extract on the serum calcium level are additive and it would appear that the two treatments do not potentiate each other

a Effect of weight loss

After the change in diet from solid food to glucose in saline solution the rats consistently lost weight (see Table VII) The mean losses for the first 3 days before control and

TABLE VIII Effect of parathyroid extract (PTE) on the serum calcium of vitamin D-deficient rats consuming different diets

Mean ± standard error Age and weight were recorded 1 2 or 3 days before the first injection The rats received the experimental diets (high protein in experiment 38 and low protein in experiment 41) from time of weaning and throughout the period of observation The experimental rats were given injections of 200 and 100 units parathyroid extract approximately 24 and 6 hours respectively before bleeding The control rats received comparable injections of physiological saline solution Rats in experiment 41 were also bled 1 day before the first injection was given

Exp and group	Age (days)	Weight (g)	No rats	Serum calcium (mg/100 ml) Before treatment	After treatment	Difference
38 PTE	45	102±4	17	—	8 77±0 27	—
38 Control	45	101±3	18	—	7 39±0 29	—
41 PTE	47	81±2	14	5 65±0 19	7 35±0 20	1 70±0 15
41 Control	47	78±2	13	5 80±0 17	6 29±0 16	0 42±0 10

parathyroid extract injections were given were essentially the same for groups I and II Unfortunately rats in Group III and IV were only weighed at the end of 4 days It is seen that the high 4-day figure for Group I is almost entirely due to heavy weight loss in the males during the last day The reason for this deviation is unknown but the importance of it is doubtful since males and females did not differ much in the other groups Although weight loss was an undesirable effect of the liquid diet these figures indicate that it was a fairly constant factor It was consequently assumed that the weight loss did not materially affect the relative response of the serum calcium level to the different treatments described above

b Effect of diet

Confirmation of the positive response to parathyroid extract in the absence of vitamin D was sought under different conditions in exp 38 The rats were fed the high protein diet throughout the experiment and they were bled only at the end of the extract treatment (see Table VIII) The age weight dietary pre treatment and the experimental procedure were otherwise essentially the same as for groups I and II in exp 37 Table VIII shows that there was a statistically significant ($P < 0.01$) difference of approximately 1 4 mg/100 ml in the serum calcium level between control and parathyroid extract injected rats Consequently under these conditions as well parathyroid extract brought about a rise in the serum calcium of vitamin D-deficient rats

In an attempt to resolve the difference in response to parathyroid extract in rats between the present investigation and that of Harrison et al (1958) the same diet (low protein) as that used by these workers was fed to rats from time of weaning in exp 41 The mothers of these rats had been given skimmed milk instead of whole milk from the 7th day of lactation as an added precaution against the transfer of even minute amounts

TABLE IX. Effect of parathyroidectomy (PTX) and sham operation (Sham) on the serum calcium of vitamin D-deficient young rats

Mean ± standard error. Age and weight were recorded 1, 2 or 3 days before time of the operation. The operated rats represent that fraction of a larger group (35 rats in both cases) which had the lowest serum calcium levels. The high protein diet was fed to all rats throughout the experiment. The calcium content of the diet in experiment 38 was reduced to 0.5% 20 days before the operation.

Exp and group	Age (days)	Weight (g)	No rats	Serum calcium (mg/100 ml): Before operation, 29 days	2 days	After operation	Individual difference
39 Sham	48	106 ± 6	6	—	8.18 ± 0.21	7.81 ± 0.35	−0.38 ± 0.24
39 PTX	48	115 ± 7	6	—	8.03 ± 0.28	5.57 ± 0.39	−2.47 ± 0.36
38 PTX	76	151 ± 6	7	6.23 ± 0.20	6.65 ± 0.16	3.83 ± 0.06	−2.87 ± 0.13

5 hours after the operation
7 hours after the operation

of vitamin D through the mothers milk. At the end of 3 weeks pre-treatment the rats were bled, treated with parathyroid extract or saline and bled again as in exp. 37. Table VIII shows that the initial serum calcium level for both groups was far lower than in the earlier experiments. *The serum calcium rise after parathyroid treatment* was highly significant ($P < 0.001$) and was more than 4 times as high as the rise after control treatment, the latter rise being of questionable significance. Whether or not the low initial serum calcium level is the result of a higher degree of vitamin D deficiency, these results show that parathyroid extract may be active also in the face of severe hypocalcemia.

c. Effect of sex

Since rats of both sexes were used in all experiments, *it was possible to test statistically* the influence of sex on the serum calcium response to parathyroid extract. The results from groups I and II (control and PTE) from exp. 37 and 41 were used in an analysis of variance of a 3-way classification with interactions. The null hypothesis was: inter action between parathyroid extract and sex is equal to zero. (Additivity is equivalent to zero interaction.) The calculated F (variance ratio) value with 1 and 32 degrees of freedom was 0.20 while the critical value at the 5% level is 4.15. Consequently the present data give no reason to believe that males and females respond differently to parathyroid extract.

Response to parathyroidectomy

In general, results obtained after injection of an impure extract of an endocrine gland require more cautious evaluation than observations made after the removal of the same gland. Additional experiments were therefore carried out with rats before and after parathyroidectomy. In both experiments (Table IX) the rats having the lowest serum

calcium levels were selected so as to give added assurance of a high degree of vitamin D deficiency In exp 39 7 week-old rats were bled both 2 days before and 5 hours after parathyroidectomy or a comparable sham operation The results showed a highly significant ($P < 0.001$) decrease of almost 2 5 mg/100 ml in the serum calcium in the parathyroidectomized animals while there was essentially no change in the control rats

In exp 38 all 7 rats were bled on 3 occasions 29 and 2 days before and 7 hours after parathyroidectomy The initial serum calcium levels were remarkably constant in spite of the calcium content of the diet having accidentally been decreased from approximately 0 7 to 0 5 per cent 20 days before the operation The reason for the difference in the initial serum calcium levels between the 2 experiments is not known As soon as 7 hours after the removal of the parathyroids very severe hypocalcemia was found the decrease in serum calcium being almost 3 mg/100 ml This decrease is significant by t test at $P < 0.001$ A very similar response was obtained in adult rats by Gran (1960 b) In view of the marked and rapid nature of the decrease in serum calcium and in view of the significant decrease observed in the control group in exp 39 it seems highly probable that the decrease was indeed due to the removal of the parathyroid glands even though sham operated controls were not included in the experiment Since removal of the source of parathyroid hormone resulted in a prompt fall in serum calcium the hormone previously secreted by the intact glands must have been responsible for maintaining the higher serum calcium level

After this work was carried out Hirsch Gauthier and Munson (1963) published a report showing that the fall in serum calcium subsequent to parathyroidectomy by cautery is not entirely due to the removal of the parathyroid hormone source but apparently also to the action of a hypocalcemic substance released from the thyroid gland Among the parathyroidectomized rats in exp 39 the occurrence of tetany in 5 rats and death of 3 rats the day after the operation indicates that there was a long lasting serum calcium depression which probably could only be ascribed to removal of the calcium mobilizing hormone Thus further evidence has been obtained for the hypothesis that parathyroid hormone is active also in rats with a high degree of vitamin D deficiency

Discussion

Parathyroid action during vitamin D deficiency

Evidence for an effect of parathyroid hormone on the serum calcium level has been obtained in vitamin D-deficient rats by subjecting them to injections of parathyroid extract or parathyroidectomy in several different experiments Since the rats were practically free from vitamin D this evidence could be interpreted to mean that either the action of the hormone is completely or partially independent of vitamin D or the action requires only traces of the vitamin If the latter were true one would expect the present rats to be less vitamin D deficient than the rats used by investigators who failed to obtain a response from moderate doses of parathyroid extract An appraisal of the criteria for vitamin D deficiency used by different authors will be instrumental in shedding light on the question of the relative vitamin D status of the present rats

In one report (Harrison et al 1958) the evidence for vitamin D deficiency was reduced concentrations of citrate in bone and serum normal serum phosphorus levels and hypocalcemia Of these only the last was measured in the present investigation and it was found to be of the same severity when the low protein diet was used in both investigations (exp 41 Table VIII) The dietary calcium and phosphorus contents in

this experiment were by analysis 0 61 and 0 67 per cent respectively as against 0 7 and 0 56 per cent respectively as reported by Harrison et al (1958) This difference in dietary composition is probably of negligible importance for the serum calcium level (Steenbock and Herting 1955) It thus seems reasonable to assume that in these two cases equal degrees of hypocalcemia indicate equal degrees of vitamin D deficiency In addition the present rats could be assumed to contain no more vitamin D than do rachitic rats prior to their being assayed for vitamin D by a curative bio-assay method (see *Criteria for vitamin D deficiency*) The only possible sources of vitamin D in the diet of the stock rats (the mothers of the present rats and of the bio-assay rats) are 3—4 g portions of cow s liver twice a month *until time of delivery and whole milk ad libitum* during the 2 first weeks of lactation only Since all experiments were carried out during the winter months the mothers vitamin D supply from the milk (approximately 0 2 I U daily) was undoubtedly too low to provide the offspring with more than traces of *the vitamin The body content of vitamin D would be further reduced during the sub*sequent 3 week period of rapid growth

Rasmussen et al (1963) report that they employed young rats obtained commercially *from* colonies maintained on diet containing minimal quantities of vitamin D The specific criterion for vitamin D deficiency was a lack of growth when the young rats consumed a vitamin D free purified diet When the diet contained 0 47 per cent calcium and 0 3 per cent phosphorus the serum calcium levels were 6 9 and 5 2 mg 100 ml respectively in two groups at the end of 3 weeks thus indicating the existence of approximately the same degree of hypocalcemia as was found by Harrison et al (1958) and in the present study (Table V and VIII)

It should be apparent from this discussion that the methods used by the present *author satisfy the requirements emphasized by* Rasmussen et al (1963) *for procuring* vitamin D-deficient rats namely maintaining rats for generations on diets containing minimal amounts of vitamin D and employing standardized purified diets

The fact that Rasmussen et al (1963) were able to raise the serum calcium in vitamin D-deficient rats by injecting massive doses (2 000 units) of parathyroid extract *is* according to the present author indicative of the idea that vitamin D is not essential for the calcium mobilizing action of parathyroid hormone in rats However this idea is apparently not supported by other results obtained by Rasmussen et al (1963) They showed that only 0 05 I U vitamin D daily for 6 days was sufficient to maintain the serum calcium at approximately 7 4 mg/100 ml until parathyroidectomy within 4 5 hours brought the level down to 4 6 mg/100 ml In the vitamin D-deficient rats on the other hand the serum calcium level of 4 9 mg/100 ml was unaffected by the same operation

Since differences between investigations in the degree of vitamin D deficiency probably were negligible other explanations must be sought for the discrepancy between the *present results and those of* Rasmussen et al (1963) and Harrison et al (1958) The latter group of investigators used only male rats as against rats of both sexes in the present work However sex was not found to have any effect on the response to parathyroid extract in the present investigation Finally there was a difference in the strain of rats used and since there was no opportunity *for testing other strains in this laboratory* the possibility remains that rat strains differ with regard to degree of parathyroid dependence on vitamin D

The present data are in agreement with those from previous work with adult vitamin D-deficient rats in this laboratory (Toverud 1963) It was shown that *the serum calcium*

level 6 and 12 days after parathyroidectomy by cautery was approximately 5 mg/100 ml while the pre operative level was approximately 9 mg/100 ml A clear return of the serum calcium to and beond the pre operative level was subsequently obtained with treatment with parathyroid extract

Although the conclusion reached by Marnay and Raoul (1959) and Marnay (1961) is the same as that of Harrison et al (1958) the results obtained by Marnay (1961) in one experiment (a serum calcium increase of 0 54 ± 0 05 mg/100 ml) may have indicated the presence of a small response to parathyroid extract in vitamin D-deficient parathyroidectomized rats

Nichols Schartum and Vaes (1963) were able to illustrate parathyroid action with vitamin D-deficient mice in two ways After injecting parathyroid extract these authors found an increase in the serum levels of calcium and phosphorus as well as an increase in passive (thermodynamic) solubility of bone mineral the latter observation stemming from experiments with untreated and heat inactivated bone samples incubated *in vitro*

The present results seem to warrant the general conclusion that in the special strain of rats maintained at this laboratory the calcium mobilizing action of parathyroid hormone is either independent of the presence of vitamin D or dependent on such minute amounts of the vitamin as may be present in severely rachitic rats

Effect of vitamin D on the serum calcium

The present results strongly indicate that vitamin D administered in a moderate dose (100—170 I U per 100 g body weight) can stimulate a rise in the serum calcium of rats deprived of either the parathyroid glands or of the dietary supply of calcium In the former case (Table III) the rise was probably due to the intestinal action of vitamin D as well as its calcium mobilizing action

Carlsson and Lindquist (1955) observed that in 46-day-old rats the intestinal effect of vitamin D was maximal after a single dose of only 10 I U while at least 100 I U were required for maximum effect on the serum calcium level Both in their study and in that of Harrison et al (1958) the increase in serum calcium 3—5 days after the administration of 100 I U vitamin D was 2—3 times greater than that observed in the present exp 37 (Table V) The reason for this difference is not readily apparent although differences in the state of the bone mineral may be important In the case of the work of Harrison et al (1958) it could be argued that the effect was partly due to the intestinal action of vitamin D since the rats continued ingesting moderate amounts of calcium

According to Nicolaysen (1937) the increased net absorption of calcium obtained by vitamin D treatment of vitamin D-deficient young rats varied between 1 and 2 mg daily whether the calcium intake was 2 4 mg or as low as 0 2 mg However for the lowest intakes net absorption was negative also after the vitamin D treatment In the present exp 37 the calcium intake from the glucose solution was presumably within this range or lower At such low intakes treatment with vitamin D will mainly affect the re absorption of digestive juice calcium If the secretion of these juices was low because of the non stimulating nature of the protein free and liquid diet and if the calcium concentration of the juices was low because of hypocalcemia (Gran 1960 a) the increase in net absorption due to vitamin D would probably have been even lower than Nicolaysen s finding of 1—2 mg Since calcium absorption probably was minimal if not negligible it seems reasonable to assume that the serum calcium rise was entirely due to the action of vitamin D on bone or another non intestinal end-organ It should be

remembered that the purpose of employing the glucose in-saline diet was *not to eliminate* absorption of calcium entirely but to prevent the deposition of significant amounts of bone mineral under the influence of vitamin D (see below Parathyroid vitamin D interaction)

The mechanism of the calcium mobilizing effect of vitamin D remains obscure However Nichols et al (1963) have made the interesting observation that vitamin D administered to vitamin D-deficient mice 3 days before sacrifice increased the solubility of bone samples incubated *in vitro* *The effect was partly due to increased passive solubility* and was therefore similar to that of parathyroid hormone

Parathyroid vitamin D interaction

There are two possible equally plausible explanations for the failure to find evidence for non additivity (mutual potentiation) of the effects of parathyroid extract and vitamin D *1) There was some degree of potentiation* but it was too small to be detected in the present experiment 2) The effects were additive It is entirely possible that a potentiating effect of vitamin D could have been demonstrated if the parathyroid extract had been given in a lower dose so that the final serum calcium level after the combined treatment would be lower than it was in this experiment The reason for this suggestion is that when the serum calcium approaches the normal level or passes it as happened in several rats in Group IV (Table V) homeostatic mechanisms aimed at lowering the serum calcium would presumably come into play and impede the rise stimulated by the treatment

A series of experiments with different combinations of doses and times of administration of the hormone and the vitamin would be needed to clarify the problem of their interaction

The most likely mechanism by which vitamin D could render a dose of parathyroid extract more effective is probably that of stimulating the absorption and deposition of increased amounts of calcium of dietary origin Long term vitamin D treatment will undoubtedly increase the amount of stable bone salt available for mobilization by parathyroid hormone If in addition the solubility of this bone salt is increased through an action of vitamin D (see above) one would expect parathyroid hormone to appear more effective in mobilizing calcium than it would in the vitamin D-deficient rat But even a single dose of vitamin D may have a similar effect when given prior to the hormone provided parathyroid hormone in the rapidly growing animal can mobilize bone deposited within the last 24 hours as suggested by Bronner (1961) This type of interaction the importance of which remains unknown was at any rate avoided in the present experiment The purpose of the glucose in saline diet was to ensure that the amount *of calcium absorbed and deposited in the skeleton in response to the vitamin D treatment* would be minute and negligible compared to the total skeletal content of calcium available for mobilization

The possibility of an interaction of the effects of the two factors on the renal handling of calcium and its importance for the serum calcium level can neither be ruled out nor discussed in the light of present knowledge

In conclusion it may be said that the present results are consistent with the hypothesis that the effects of parathyroid *extract and* vitamin D on the serum calcium are additive but the results are inadequate to rule out the possibility of an interaction between the effects

Parathyroid action in rachitic children

Neither Jonxis (1961) nor Steendijk (1964) were able to significantly raise the serum calcium level in severely rachitic children by injecting parathyroid extract Only after prolonged treatment with vitamin D was a response obtained in one patient after administration of 700 units parathyroid extract (Eli Lilly Co) over 5 days (Steendijk 1964) However there is also evidence to the contrary namely that the hormone is active in rickets 1) The serum calcium level in primary vitamin D deficient rickets is usually normal or slightly subnormal 2) In cases of rickets and osteomalacia (Liu et al 1941) with slight hypocalcemia and marked hypophosphatemia vitamin D treatment results in only a slow and delayed return of the serum calcium level but a rapid return of phosphate to the normal level (Steendijk 1962) 3) Stalder et al (1962) have shown that a rachitic child could respond to vitamin D treatment while on a calcium free diet by an immediate drop in urinary phosphate excretion and an increase in renal tubular phosphate reabsorption (in per cent of filtered phosphate) in spite of a steadily rising serum phosphate level If one of the effects of vitamin D in this case had been the re-establishment of end-organ responsiveness to parathyroid hormone the well-established *phosphaturic effect of this hormone would have counteracted the observed* urinary changes Relative hyperphosphaturia and hypophosphatemia do not fit the concept of parathyroid hormone inactivity in the absence of vitamin D and these phosphate abnormalities may actually be partly explained by assuming the existence of a hyperparathyroid state secondary to rickets (Rasmussen and Reifenstein 1962)

The discrepancy between these findings may possibly be explained by assuming that *the small amount of bone salt present in the severely rachitic child and perhaps also the* lower solubility (see above Effect of vitamin D on the serum calcium) of the bone make it necessary to inject a very high dose of parathyroid extract to obtain a measurable effect on the serum calcium

The author wishes to express his gratitude to D Paul L. Munson for advice on the design of experiments to Mr Dan Wo en fo advice on statistical treatment, and to Miss U M Kristoffersen for valuable technical assistance The autho greatly app eciates the gift of parathyro d extract from the Eli Lilly Co through the courtesy of Dr Otto K. Behrens

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Studies on the Elimination of Exogenous Lipids from the Blood Stream Determination and Separation of the Plasma Triglycerides after Single Injection of a Fat Emulsion in Man

By

DAG HALLBERG

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Abstract

Hallberg D *Studies on the el minaton f exo_enous l pids f om the blo d stream D term n tion and sep tio i of the plasma tri lycerides after si le injection f a fat mulsion in man* Acta phys ol scand 1964 6? 407—421 — Single injection of a fat emulsion into the blood stream in man resulted into a complex elim nationcurve when total triglyceride (TG) concentration in pla ma was followed The complex ty of the curve was studied by separation of the plasma TG e ther by centrifugation or by a density gradient method with polyvinylpyrrol done (PVP) Centri fugation of plasma at 48 6 × 10 G minutes d d not solve the complex curves The PVP m thod gave three TG fractions fat emulsion n the top fraction and endogenous plasma TG in the botto n and middle fraction Elim nation curves obtained with the PVP method v ere compos site The TG concentration in the top fract on wh ch was zero in fasting plasma ncreased after injection of the emuls on and then dec eased This decrease could be resolved nto a l near phase at high TG concentration and an exponential phase at lower concentrations The curve for the bottom f a tion showed a slo ly increasing concentration and that for the m ddle f act on sho ved a r pid increase followed by a decreas The complex appearance of the curve without fract onat on of the TG depends on these two latt lipid fractions which change in conce itration during the elim nation The diff culties n interpreting the el mination curves obtained by th entrifugat on method was w th the gradient shown to depend on heter ogene ty in the top TG fract on obta ned by centrifugat on

Interest has been devoted to lipid emulsions for intravenous use because of advantages in clinical practice This interest has among other things been focused on the metabolism of infused lipids

Carlson and Hallberg (1963) described the curve for the elimination of a fat emulsion (Intralipid[K]) from the blood stream in fasting dogs. This curve was found to be complex. However, it was possible to resolve it into two phases of elimination: one linear elimination (zero order kinetics) at high TG[1] concentration (above 1 mmol of TG/l blood) and one exponential elimination (first order kinetics) at lower concentrations.

The curve for the elimination of this fat emulsion in man was found to be still more complex than in dogs. This complexity has here been studied by separating plasma TG into fractions by centrifugation and by a density gradient method.

In 1962 Gordis described a density gradient method with polyvinylpyrrolidone (PVP) and demonstrated two kinds of particulate fat in alimentary blood. This method has here been tested for use in the separation of plasma TG for determination of the elimination of the fat emulsion from the blood in man.

Material and methods

Procedures

In vivo studies. After an overnight fast healthy individuals between 20 and 40 years old were injected i.v. with 0.3 g fat/kg b. w. as Intralipid[®] 20 (20 per cent soya bean oil, 1.2 per cent lecithin and 2.5 per cent glycerol. Schubert and Wretlind 1961). The injection time was below two minutes. Blood samples were drawn at different intervals from a permanent needle in an arm vein. The blood was taken into chilled heparinized tubes and stored in ice water not longer than 4 hours before extraction. This interval did not influence the results. After the test all samples were centrifuged to separate off plasma at 400 C min (according to Dole and Hamlin 1962) (Doctor centrifuge, A. B. Wifug, Stockholm, Sweden).

Centrifugal separation

Plasma samples were centrifuged for 30 min in the 40.3 rotor of a Spinco Model L ultracentrifuge. The plastic tubes were cut in the middle with a Spinco tube slicer and samples from the bottom fraction were extracted for determination of TG concentration.

PVP gradient separation

A density gradient with PVP (Plasdone, average m. w. 40 000, obtained from General Aniline and Film Co., New York, USA) was made as described by Gordis (1962) by displacing fluid of continually increasing PVP concentration from a mixing bottle into the bottom of a plastic test tube. By using tubes smaller than recommended the gradient will be steeper and the experience was that the different layers will be more distinct in 10 ml tubes (Spinco 5/8 — 2 1/2) than in 20 ml tubes.

2 ml of plasma was mixed with 0.5 ml 25 PVP solution in 10 NaCl with 0.01 EDTA (ethylene diamine tetraacetate) and 200 mg powdered NaCl was added. 1 ml of this mixture was extracted for determination of TG concentration and 1 ml layered with a syringe and a thin needle in the bottom of a tube containing the gradient. The TG were allowed to separate for about 17 hrs at 37 C.

After this time there were three layers in the gradient. The tubes were sliced with a tube slicer about 1.5 cm from the top and the top fraction was harvested. Then the whole bottom fraction was quantitatively aspirated with a thin needle pierced through the bottom of the tube. The same TG concentration was found in the bottom fraction when only 0.5 ml was aspirated as when the whole fraction (1 ml) was aspirated. The rest (middle fraction) was collected quantitatively. TG determinations were performed on the three fractions.

The method was tested in *in vitro* studies designed to separate fat emulsion TG from fasting plasma TG and in *in vivo* studies.

[1] Abbreviations: TG = Triglyceride(s), PVP = Polyvinylpyrrolidone.
[2] The emulsion was kindly supplied by A. B. Vitrum, Stockholm, Sweden.

TABLE I TG concentration (mmole/l) in vitro in fasting plasma with and without fat emulsion (Intralipid'®) before and after centrifugation at different G min Each value is the mean of a double extraction

Pl = plasma Em = fat emulsion

No	Sample	TG conc before centrifugation	TG conc in the bottom fraction after centrifugation				
			5.4×10^4 G min	21.5×10 G min	48.6×10 G min	86.0×10 G min	344×10 G min
1	Em+saline	16.00	0.85	0.28	0.20	0.16	0.06
2	Pl	0.64	0.64	0.66	0.66	0.60	—
3	Pl	0.95	0.94	0.80	0.76	—	—
4	Pl	0.51	—	0.45	0.47	0.41	—
	Pl+Em	2.50	—	0.60	0.48	0.43	—
5	Pl	0.76	0.78	0.78	0.71	0.68	0.50
	Pl+Em	10.48	1.53	1.06	0.92	0.79	0.56
6	Pl	0.64	0.57	0.55	0.50	0.43	0.41
	Pl+Em	16.70	1.22	0.88	0.67	0.67	0.52
7	Pl	1.38	1.35	1.41	1.28	—	—
	Pl+Em	5.40	2.00	1.59	1.40	—	—
8	Pl	1.04	—	1.00	1.04	—	—
	Pl+Em	7.90	—	1.23	1.10	—	—
	Pl+Em	7.90	—	1.12	1.03	—	—
9	Pl	1.38	—	—	1.27	—	—
	Pl+Em	7.15	—	—	1.40	—	—
10	Pl	1.37	—	—	—	—	1.02
	Pl+Em	4.24	—	—	—	—	0.96
11	Pl	1.42	—	—	—	—	1.13
	Pl+Em	8.00	—	—	—	—	1.09
12	Pl	1.52	—	—	1.32	1.37	—
	Pl+Em	8.30	—	—	1.55	1.51	1.36

Analytical methods

All TG determinations were done according to Carlson's (1963) method The mean of triple determinations on each extract are given The blank values of the method were not affected by PVP material or by the different fractions in the gradient or by analyses on different amounts of the chloroform extract

Analytical errors

The errors of the analyses were calculated from duplicate determinations according to the formula $S = \sqrt{\frac{\sum d^2}{2n}}$

(d = difference between two duplicates and n = number of duplicates)

The presence of partial glycerides was tested by thin layer chromatography After the addition of methanol to 5 th chloroform extract was passed through a column of silic acid After concentration the samples and a mixture of reference substances consisting of mono-d and triglycerides were put on the plates No detectable partial glycerides were found in the samples tested

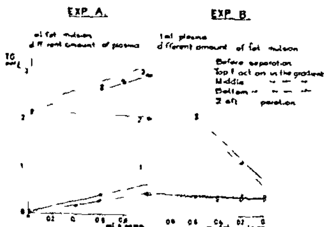


Fig. 1. Triglyceride concentration (mmole/l) of fat emulsion and plasma before and after separation with EVP gradient into three fractions (top, middle and bottom) and their sum. Exp. A. Separation of a constant amount of fat emulsion added with different amounts of plasma.
Exp. B. Separation of a constant amount of plasma added with different amounts of fat emulsion.

1. The error for a single determination of plasma TG was ± 0.05 mmol/L (n = 250)
2. The error for a single determination of plasma TG *in the bottom fraction after centrifugation* was ± 0.05 mmole/L (n = 100)
3. The error for a single determination of plasma TG separated in the EVP gradient was
   0.05 mmole/L for the top fraction (n = 26)
   ± 0.09 mmole/L for the middle fraction (n = 31)
   ± 0.08 mmole/L for the bottom fraction (n = 31)
4. The mean recovery of TG after gradient separation in vitro and in vivo was 103 with a standard deviation of 11 (n = 146)

Calculations

After centrifugation the amount of TG in the top fraction was calculated as the difference between the precentrifugation value and the amount of TG in the bottom fraction.

After separation of plasma into 3 fractions in the EVP gradient the recovery was calculated and expressed as a percentage of the amount of TG present in the unseparated plasma.

The statistical methods used were according to Snedecor (1957).

Results

In vitro studies

Centrifugation. The results from the centrifugal separation of fasting plasma with and without addition of Intralipid® at different G min (Dole and Hamlin 1962) are presented in Table I.

It was found that the TG concentration in the bottom fraction of fasting plasma without added emulsion decreased slightly with increasing G-min.

The TG of emulsion added to saline was found to have almost disappeared from the bottom fraction at 48.6 × 10⁴ G min. When plasma with added emulsion was centrifuged at 48.6 × 10⁴ G min the TG concentration found in the bottom fraction was close to the concentration of the fasting plasma. However in plasma alone

TABLE II TG-concentration (mmole/l) in the different fractions after PVP gradient separation of plasma with and without adding fat emulsion in vitro

Pl = plasma Em = fat emulsion

No	Sample	TG conc before separation	TG conc. after separation				Recovery in
			Top fraction	Middle fraction	Bottom fraction	Σ	
1	Pl	1 14	0 11	0 53	0 67	1 31	115
	Em	1 99	1 77	0 17	0 06	2 00	100
	Pl+Em	3 22	1 70	0 55	0 67	2 92	91
2	Pl	0 68	0 16	0 30	0 37	0 83	121
	Em	2 18	2 16	0 19	0 09	2 44	112
	Pl+Em	2 75	2 03	0 41	0 41	2 85	103
3	Pl	0 87	0 09	0 48	0 34	0 91	104
	Em	3 70	3 62	0 02	0 00	3 64	98
	Pl+Em	4 64	3 33	0 55	0 40	4 28	92
4	Pl	0 73	0 00	0 41	0 45	0 86	118
	Pl+Em	4 71	4 02	0 59	0 53	5 14	120
5	Pl	0 62	0 08	0 15	0 41	0 64	103
	Pl+Em	2 42	2 01	0 19	0 44	2 64	110
6	Pl	0 75	0 06	0 07	0 57	0 70	94
	Pl+Em	2 50	1 77	0 10	0 56	2 43	97
7	Pl	0 69	0 03	0 18	0 51	0 72	104
	Pl+Em	4 03	3 48	0 20	0 56	4 24	105
8	Pl	0 64	0 18	0 00	0 52	0 70	109
	Pl+Em	6 14	5 39	0 07	0 61	6 07	99
9	Pl	0 78	0 03	0 17	0 62	0 82	105
	Pl+Em	2 05	1 26	0 20	0 61	2 07	100
10	Pl	0 86	0 31	0 22	0 28	0 81	94
	Pl+Em	4 20	3 29	0 23	0 42	3 94	94
Mean	Pl (1)	0 78	0 11	0 25	0 47	0 83	106 7
1–10	Pl+Em (2)	3 67	2 83	0 31	0 52	3 66	101 1
Δ	(2)—(1)	2 89	2 72	0 06	0 05	2 83	—

centrifuged under identical conditions the concentration of TG of the bottom fraction had in some instances decreased slightly The 48 6   10 G-min was considered to be the condition that least affected plasma TG concentration and most completely separated added fat emulsion TG from the bottom layer and was therefore used for the *in vivo* experiments

PVP method

Two different types of experiments A and B were performed

A Increasing amounts of fasting human plasma were added to constant amounts of diluted fat emulsion (about 1   )

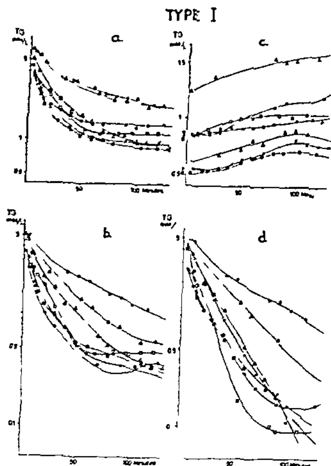


Fig 2 Triglyceride (TG) concentration (mmole/l) in plasma in 7 subjects during elimination of i v injected fat emulsion (0 3 g fat/kg) (Type I curves)

a. Total TG concentration in plasma on a semilogarithmic scale

b Total TG concentration minus fasting TG concentration on a semi logarithmic scale

c. TG concentration in the bottom fraction after centrifugation (48 G × 10 G min) on a linear scale

d Total TG concentration minus bottom TG concentration after centrifugation on a semi logarithmic scale

B Increasing amounts of emulsion were added to constant amounts of plasma The volumes were equilibrated by adding saline Data from a typical experiment are given in Fig 1

In A the fat emulsion was quantitatively recovered in the top fraction after gradient separation. The addition of plasma did not influence the recovery in the top fraction

In B the main part of plasma TG was found in the bottom and middle fraction after gradient separation The addition of increasing amounts of lipid emulsion did not

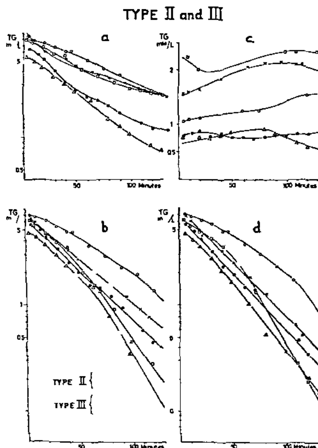


Fig 3 Triglyceride (TG) concentration (mmole/l) in plasma in 5 subjects during elimination of i v injected fat emulsion (0 3 g fat/kg) (Type II and III curves)
a. Total TG concentration in plasma on a semilogarithmic scale
b Total TG concentration minus fasting TG concentration on a semilogarithmic scale.
c TG concentration in bottom fraction after centrifugation (48 6 × 10⁴ G-min) on a linear scale
d Total TG concentration minus bottom TG concentration after centrifugation on a semilogarithmic scale

alter the TG concentration in the bottom and middle fraction to any considerable degree

The PVP gradient separation was applied on fasting human plasma with and without addition of fat emulsion The results are given in Table II

Fasting plasma separated into three fractions the main part of TG was found in the bottom and middle fraction and only 14 in the top Added fat emulsion was mainly found in the top fraction and there was a slight increase in TG concentration

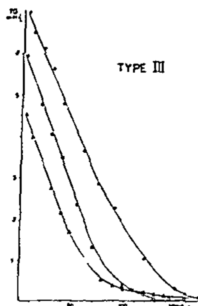


Fig 4 Calculated top fraction TG concentration (mmole/l) after centrifugation (48 6 × 10⁴ G min) of plasma from 3 subjects during elimination of intravenously injected fat emulsion (0 3 g fat/kg) on a linear scal (same data as Type III in Fig 3 d)

in the bottom (0 47 → 0 52 mmole/l) and middle (0 25 → 0 30 mmole/l) fraction This increase was statistically significant

In vivo studies

Centrifugation The curves obtained in man after injection of the fat emulsion were classified into 3 types according to the appearance in semilogaritmic plot of the curve for the concentration of total plasma TG minus the fasting value (Carlson and Hallberg 1963)

Type I The curve was mostly concave upwards

Type II An almost linear curve

Type III The curve was mostly convex upwards

The elimination curves for 12 individuals are compiled in Fig 2 and 3 7 of these curves were classified as Type I (Fig 2) 2 as Type II and 3 as Type III (Fig 3)

Fig 2 and 3 give the total TG concentration (a) total TG concentration minus the *fasting TG concentration* (b) *the TG concentration in the bottom fraction after* centrifugation (c) and total TG concentration minus the bottom TG concentration (= the calculated top fraction after centrifugation) (d)

The curves for total TG concentration (Fig 2a and 3a) are all complex and do not allow simple mathematical interpretation The curves for total TG concentration minus the fasting TG concentration are complex for Type I (Fig 2b) seem to be exponential in Type II according to the definition (Fig 3b) and in Type III composite of a linear phase at high concentration and perhaps an exponential phase at lower concentration (Fig 3b and 4)

The curves for the TG concentration in the bottom fraction show with time mostly increasing values in Type I (Fig 2c) and variable concentrations in Type *II* and *III* curves (Fig 3c)

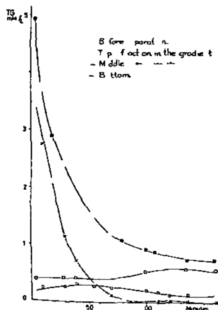


Fig 5a

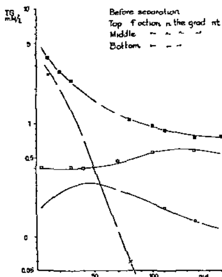


Fig 5b

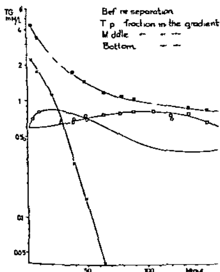


Fig 6a

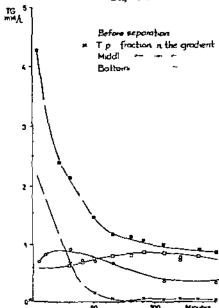


Fig 6b

Fig 5 and 6 a Elimination from the blood stream of injected fat emulsion (0 3 g fat/kg) in 2 subjects The triglyceride concentrations are given for plasma before and after separation in the PVP *gradient into 3 fractions*
b The same data as in a but on a semi logarithmic scale.

TABLE III TC concentration (mmole/l) in the top fraction of plasma taken at intervals after the injection of fat emulsion (0 3 g fat/kg) i v into one subj ct. Plasma was separated by centrifugation (48 6 10⁴ C min) and by PVP gradient

	Time/minutes											
	0	5	10	20	40	60	70	80	90	100	110	120
tc	0	4 15	3 22	1 90	1 07	0.51	0 37	0 27	0 18	0 05	0 07	0 05
tg	0 15	3 65	2 46	1 25	0 19	0 12	0 11	0 07	0 06	0 04	0	0
tc tg	0	0 50	0 76	0 65	0 88	0 39	0 26	0 20	0 12	0 01	0 07	0 05

tc = TC concentration in top fraction after centrifugation of plasma (calculated see under Methods)
tg = TC concentration in the top fraction of plasma separated by the PVP gradient.
tc tg = The difference between the two top fractions (Centrifugation gradient)

TABLE V TC concentration (mmole/l) in plasma at intervals after injection of fat emulsion (48 6 × 10 G min) and by the PVP grad ent (See Methods)

	Subject I					
	Time/minutes					
	0	5	10	20	30	40
tc	0 05	4 89	4 65	4 12	2 55	2 32
tg	0	5 14	4 72	4 05	2 70	2 48
bc	0 85	0 91	0 85	0 90	0 87	0 92
bg	0 42	0 83	0 77	0 78	0 76	0 86
mg	0	0	0 01	0 19	—	0
tc tg	0 05	−0 25	−0 07	0 07	−0 15	−0 16

	Subject II					
	Time/minutes					
	0	6	10	20	30	40
tc	0 03	3 22	1 90	1 44	1 00	0 57
tg	—	3 10	1 75	0 74	0 51	0 43
bc	0 55	0 56	0 56	0 53	0 55	0 62
bg	—	0 50	0 57	0 55	0 49	0 55
mg	—	0 18	0 14	0 68	0 55	0 21
tc tg	—	0 12	0 15	0 70	0 49	0 14

tc = top fraction after centrifugation
tg = gradient top fraction
mg = calculated m ddle faction in the gradient

TABLE IV TG concentrat on (mmole/l) in plasma at intervals after inject on of fat emulsion (0 3 g fat/kg) v in one subject The top fraction after centrifugation (48 6 × 10 G m n) was isolated and layered in the bottom of the dens ty gradient tube

	Time/minutes												
	0	4	10	17	25	35	45	65	80	90	100	110	124
TG	1 37	6 36	4 86	4 56	3 66	3 37	3 28	2 50	2 20	2 12	2 30	1 92	1 93
tc	0 16	5 13	—	3 20	2 30	2 03	1 90	1 00	0 66	0 61	0 7?	0 3?	0 38
tg	0 10	4 45	4 11	2 61	2 30	1 41	29	0 37	0 23	0 16	0 12	0 05	0 06
tc tg	0 06	0 68	—	0 59	0	0 62	0 61	0 63	0 43	0 45	0 60	0 27	0 32

TG = Total TG concentration in plasma
tc = Calculated TG concentration in the top fraction after centrifugation of plasma
tg = TG concentrat on in the top fraction after gradient separation of the isolated cantrifugal top fraction

(0 3 g fat/kg) i v in 2 subjects Plasma was separated into fractions by centrifugat on

51	60	70	80	90	100	114	123	135
2 16	1 94	1 46	1 43	1 39	1 14	0 98	0 63	0 45
1 95	1 85	1 25	0 85	0 74	0 58	0 44	0 25	0 24
0 9?	0 93	0 94	0 96	0 94	0 99	1 0?	0 99	1 18
0 90	0 97	0 86	0 78	1 02	0 84	0 88	0 87	0 96
0 23	0 05	0 29	0 76	0 57	0 71	0 68	0 50	0 43
0 21	0 09	0 ?1	0 58	0 66	0 56	0 54	0 38	0 21

50	60	70	80	90	100	110	120	130
0 46	0 35	—	—	0 16	0 11	0 05	0 13	0 07
0.28	0 12	0 11	0 08	0 04	0	0 04	0 06	0
0 65	0 66	0 71	—	0 76	0 83	0 79	0 78	0 77
0 60	0 59	0 66	0 74	0 76	0 73	0 74	0 54	0 60
0 23	0 30	—	—	0 1?	0 ?1	0 06	0 30	0 24
0 18	0 23	—	—	0 12	0 11	0 01	0 07	0 07

bc = bottom fraction after centrif gat on
bg = bottom fr ction in th grad ent

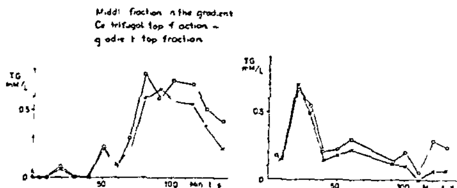


Fig 7 The difference in triglyceride concentration between the two top fractions after centrifugation (48 6 × 10 G min) and separation in the PVP gradient respectively compared with the middle fraction of the gradient Plasma samples taken at intervals during elimination of 0 3 g fat/kg fat emulsion injected i v Data from 2 subjects are given

The curves for the top fraction are for Type I (Fig 2d) still complex and for Type II (Fig 3d) still seem to be exponential For Type III (Fig 3d) the curves are of the same nature as in the plot minus the fasting TG concentration (Fig 3b) but the phases are steeper These Type III curves seem to be linear at the beginning when plotted on a linear scale (Fig 4)

PVP method

Results with the PVP method are illustrated by curves from two subjects who both belonged to Type I (see above) (Fig 5 and 6) The curves for total TG concentration are complex The curves for the top fraction (emulsion fraction) however can be resolved into a linear part at high lipid concentration and an exponential part at lower concentrations The curves for the bottom fraction show a slowly increasing TG concentration The curves for the middle fraction show a rapidly increasing concentration followed by a decrease

Comparison of the centrifugal and the PVP gradient method in vivo

Three different studies A B and C were made on plasma taken at intervals from subjects who had got 0 3 g fat/kg as a single injection

A Plasma from one subject was analysed by both methods and the sizes of the top fractions were compared (Table III) The centrifugal top fraction contained more TG than did the gradient top fraction The difference varied with time and was most pronounced 40 minutes after the injection

B Plasma from another subject was fractionated with the centrifuge The top fraction was collected and layered in the bottom of the PVP gradient After separation the gradient top fraction was analysed for TG (Table IV) There was a difference between the calculated centrifugation top and the gradient top fraction the latter being smaller This indicates heterogeneity of the TG in the centrifugation top fraction

C Plasma from two subjects was analysed by both methods and the different fractions were compared (Table V) The bottom fractions were of the same size The centrifugal top fraction was greater than the gradient top fraction. The difference was of the same order as the gradient middle fraction (Fig 7)

Discussion

"The disappearance of (fat) particles from circulation has proven easier to measure than to interpret" (cit. Dole and Hamlin 1962)

This statement can characterize our present knowledge on the elimination of exogenous[1] lipids from the blood stream in man. The scope of this investigation was to find a mathematical model for the elimination process in man. When studied by following the plasma TG concentration this process was more complex than in the dog. To get data for interpretation it was necessary to find a technique for differentiation of glycerides of exogenous and endogenous origin[1].

Two methods have been used here: centrifugal and density gradient separation of lipids.

The results with the centrifugation method indicates that the conditions for separating the TG of the Intralipid® emulsion from plasma TG must be chosen arbitrarily because there are TG in fasting plasma which flotate under the centrifugal condition needed to remove the fat emulsion from the bottom fraction. It was judged that 48.6 × 10 G min was the condition of those studied which least affected the plasma TG and at the same time most completely flotated the fat emulsion to the top.

The observed change in fasting TG concentration may belong to the heavy chylomicron fraction described by Albrink (1961) or to the secondary particle fraction of Gordis (1962). At the moment there is no information on the diurnal change in concentration of these kinds of lipoproteins. The degree of observed chang in concentration was variable in plasma from different subjects. In the in vivo experiments it was realized that this uncontrollable centrifugal change of fasting plasma TG concentration was an error making the method not ideal for elimination studies.

The PVP gradient method separated fasting plasma into fractions where the main part of the plasma TG was located in the two lower fractions. The addition of Intralipid® caused a very small increase in these two plasma fractions. Added fat emulsion was found in the top fraction. The mean recovery was 103 % after separation. The mean difference between fractions of 10 different plasmas in the gradient before and after adding fat emulsion was within the error of gradients analysed in duplicate.

With the PVP gradient it was shown that the top fraction after centrifugation of plasma from subjects given Intralipid® was heterogenous in composition and that the difference between the centrifugal and the gradient top fraction concentration changed during the elimination. This difference was of the same magnitude as and changed in parallel with the concentration in the gradient middle fraction.

There was a good agreement between the TG concentration in the bottom fraction of the gradient and that in the centrifuge indicating that most lipoproteins are in the bottom of the gradient.

[1] By exogenous lipids is here ment chylomicron from the thoracic duct and fat emulsions. Once they h e left the blood stream and if they reappear th y are ons d red to be endogenous lipids. Th f t emuls on w s used as an eas l a ailable chylomicron model. There are reasons whi h justify the comparison of chylomicrons and Intralip d® as a b ological material. They have the same rates of limination from the blood stream n dogs (Carlson and H llberg 1963). The rate constants were about the same when the were compared as substrates in l poprotein l pase react ons (Boberg and Carlson 1964). The partı le size in the electron m croscope was about the same (Hallberg and Wersall 1964).

The small increase in TG concentration in the gradient plasma fractions observed in vitro after adding fat emulsion is probably of minor importance in the method because during elimination there was a decrease in the fat emulsion fraction while the concentration in these plasma fractions increased. This fact argues against a concentration dependent error in the method.

All in vivo experiments showed that after a single injection of the fat emulsion the elimination curve for total TG concentration had a complex appearance.

Assuming that the elimination is a concentration dependent process which can be described in mathematical terms then the observed complexity would depend on disturbing lipids that appear in the plasma after the initial process of elimination.

In spite of fractionation of plasma TG in the centrifuge only a few curves could be interpreted with the same model as earlier described for dogs. If the assumption *made above is correct then there are a few possibilities to explain the complex curves.* If the disturbing lipid fraction increases in concentration during the elimination process then the curve will belong to Type I (concave upwards in semilogarithmic plot). If the endogenous TG fractions do not change in concentration then the curve will be Type II (linear on a semilogarithmic plot). If the endogenous TG concentration decreases the Type III curve will appear. There may of course also be more than one disturbing TG fraction which would further complicate the interpretation of the curve for total TG concentration. These considerations justified the typing of the centrifugal curves. The results with the centrifugal method showed that there was a disturbing lipid in the bottom fraction which mostly increased in concentration during the elimination.

However the centrifugation top fraction considered to be the emulsion fraction proved to be heterogeneous in the PVP gradient. The part of the centrifugal top that could not be found in the gradient top was found in the gradient middle fraction.

Two curves for the elimination of the fat emulsion from the blood stream are presented after analyses of plasma by the PVP gradient method. The curve for the top fraction *that represents the fat emulsion in plasma has an appearance which seems to be* identical with that found in the dog (Carlson and Hallberg). This means that the elimination process can be expressed in mathematical terms as described previously a linear phase at high TG concentration and an exponential phase at lower TG concentrations.

The complexity of the elimination curve has been shown to depend on two disturbing lipid fractions that are recovered in the bottom and middle fractions in the PVP gradient. These two fractions vary in concentration during the elimination.

With this explanation of the complexity there are two things requiring an answer.

Firstly how to explain the found linear phase in the Type III curves? (Fig 4). The middle fraction TG may be masked *within the linear phase. Secondly why was the* elimination curve in the dog found without separation of lipids into different fractions? The answer to this can be that disturbing lipid fractions in the dog have only a small change in concentration or do not exist and consequently are not observed. *Gordis Bierman* and Hamlin (1962) have demonstrated that the secondary particles (*gradient middle* fraction TG) during alimentary lipemia probably receive material from the primary particles (chylomicrons in the gradient top fraction). It is possible that the same kind of process takes place during elimination of intravenous lipid

emulsions and that the observed increase in the middle fraction consists of TG first taken up in some organ and then recirculated to the plasma in a new form

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Acta physiol scand 1964 62 422—428

From the Institute of Physiology University of Uppsala Sweden

The Disappearance of Acetic Acid and Acetate from the Cat's Stomach and its Influence on the Permeability for Hydrochloric Acid

By

G FLEMSTRÖM B FRENNING and K J ÖBRINK

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Abstract

Flemstrom G B Frenning and K J Öbrink *The disappearance of acetic acid and acetate from the cat's stomach and its influence on the permeability for hydrochloric acid* Acta physiol. scand 1964 62 422—428 — Experiments were performed on anaesthetized cats to study the diffusion of acetic acid out of a nonsecreting stomach and its effect on the gastric permeability for hydrochloric acid Acetic acid diffuses more rapidly than hydrochloric acid out of the stomach The diffusion of hydrochloric acid is increased by an instillation of acetic acid The acidity of the instilled hydrochloric acid decreases more rapidly and reaches lower end values There is also a larger initial dip in chloride concentration A mixture of sodium acetate and hydrochloric acid was found to have a similar effect on the permeability for hydrochloric acid

Since the work of Teorell (1933) on the permeability of the gastric mucosa much evidence has appeared that the regulation of the gastric juice acidity is due to a great extent to permeation of hydrogen sodium potassium and chloride ions For a review of these aspects the reader is referred to Heinz and Öbrink (1954) In 1939 Teorell also analyzed the permeability properties of a non secreting stomach for several other acids as well as for some other substances It should be noted that in his work on weak acids he showed that permeability coefficients were higher for the weaker acids

Interest in the penetration of weak acids and bases has occurred repeatedly in the literature The studies on secretion of dyes and drugs are noteworthy (see for instance Hogben *et al* 1957 Schanker *et al* 1957)

It has been observed that weak bases are accumulated in the gastric juice whereas weak acids do not enter In the pancreatic juice the opposite seems to take place This has been explained due to penetration of these weak bases or acids in undissociated

forms Diffusion is thought to take place not only between the cells but also through the lipid layers

Babkin and his school paid special attention to the effect of acetic acid on the stomach (Babkin Hebb and Krueger 1941 Babkin 1950) They showed that this acid could be a powerful stimulant for gastric mucus (Grant 1942) The rapid neutralization of acetic acid in the stomach could therefore be expected to be a result both of a high permeability coefficient and of the effect of the increased mucus secretion

The effect of acetic acid may however be much more complicated Gorbunova Lebedinskaia and Savich (1933 cited from Babkin *et al* 1941) introduced acetic acid into the main stomach of Pavlov pouch dogs and observed a decrease in the secretory response from the pouch after a test meal Babkin *et al* (1941) repeated these experiments and found that the diminution of the hydrochloric acid response occurred also after histamine stimulation They could even obtain a persistent effect of the acetic acid treatment

All the above experiments make it especially interesting to study in more detail the permeability properties of the gastric mucosa towards the acetic acid and acetate as well as the possible influence of these substances on the permeability coefficient for hydrochloric acid

The experiments to be reported are a preliminary work on these topics

Methods

Experimental animals

The experiments were performed on anaesthetized cats (chloralose urethane 1 10 v) that had been fasted for at least 17 hours After a midline incision, ligatures were tied around the cardia and pylorus care being taken not to interrupt the blood supply to the stomach A glass cannula was introduced into the pyloric portion Through this cannula a rubber tube could be inserted for filling or emptying the stomach After completion of the surgical operation the cats were left for one two or more hours during which time it could be tested whether the stomachs were at secretory arrest A test solution was then introduced and remained in the stomach for a given length of time After this period the volume was completely removed measured and re introduced after a small sample had been taken for analysis

The stomach was rinsed with the fluid to be introduced before every new experiment was started

Analytical procedures

Acidity determination 0 1 ml sample was put into 4 ml of distilled water and titrated with 0 01 M NaOH using bromthymol blue as an indicator That means that only total acidity' was determined as this indicator changes its colour between pH 6 0 and 7 6

Chloride determination was made electrometrically with a silver chloride electrode on the same samples as used for the acidity determination For titration 0 005 M $AgNO_3$ was used

Osmolarity determination was made from freezing point depression measurement with the osmometer of Advanced Instruments Inc Newton Highlands Mass U S A

Three types of experiments were performed

A Introduction of 170 mM (33[illegible] mOsm) HCl followed by introduction of 170 mM (168 mOsm) HAc in the same cat

B Consecutive experiments on the same cat with a) 170 mM HCl b) 170 mM HAc and c) 170 mM HCl

C. Consecutive experiments on the same cat with a) 170 mM HCl b) 170 mM HCl to which was added 170 m moles per liter solution of sodium acetate in powder form (483 mOsm) and c) 170 mM HCl

In all types of experiments 6 ml of the test solution were introduced

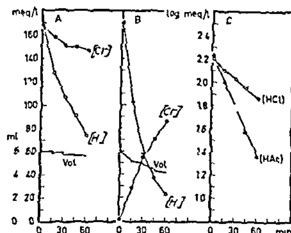


Fig 1 A typical experiment with instillation of hydrochloric acid in a cat's stomach followed by instillation of acetic acid A 6 ml of 170 mM hydrochloric acid B 6 ml of 170 mM acetic acid. C The hydrogen ion concentrations plotted on a semilog arithmic scale

TABLE I Instillation of 6 ml hydrochloric acid and acetic acid respectively in a cat's stomach and an experiment with a model where 12 ml of the test solution were allowed to diffuse against 60 ml of 170 mM sodium chloride through a cellophane membrane k = the permeability coefficient for hydrogen ions (ml min⁻¹) ΔV ml is the change in volume per 60 min ΔH (mM) = the decrease in total acidity in the first 60 min

Exp no	k_{HCl}	k_{HA}	ΔV_{HCl}	ΔV_{HAc}	ΔH_{HCl}	ΔH_{HA}
1	0 11	0 34	+ 0 5	− 1 3	117	160
2	0 06	0 18	+ 0 3	− 1 6	85	166
3	0 09	0 35	+ 0 1	− 1 8	107	168
4	0 06	0 17	0	− 1 5	95	1 8
Mean value	0 08	0 26	+ 0 2	− 1 6	101	161
Model exp	0 05	0 0?	–	–	40	17

Results

A Comparison of the permeability properties for hydrochloric acid and acetic acid in the same stomach

Six ml of 170 mM HCl were introduced into the stomach and samples taken every 15 min The resulting concentration of the hydrogen ion is seen in Fig 1A A consecutive introduction of 6 ml 170 mM HAc into the same stomach showed a similar disappearance as can also be seen in Fig 1B The disappearance rate of acetic acid was always greater than that of hydrochloric acid See Table I This table also summarizes similar experiments performed in a model where hydrochloric acid and acetic acid were allowed to diffuse against 170 mM NaCl through a cellophane membrane It

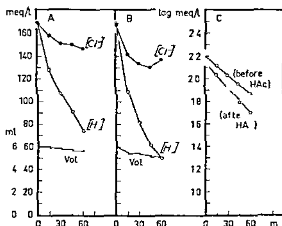


Fig 2 An experiment with two instillations of hydrochloric acid on the same cat interspaced by an instillation of acetic acid A 6 ml of 170 mM hydrochloric acid before 170 mM acetic acid B 6 ml of 170 mM hydrochloric acid after 170 mM acetic acid C The hydrogen ion concentrations plotted on a semilogarithmic scale

TABLE II Permeability coefficients (ml min^{-1}) and maximal chloride ion concentration decrease mM in instillations of hydrochloric acid in a cat's stomach before and after an instillation of acetic acid The chloride ion concentration decrease is measured as the difference between the original concentration and the lowest one Experiment no 5 is an experiment with 170 mM HCl to which was added sodium acetate in powder form to a final concentration of 170 mM instead of 170 mM HAc

Exp no	Permeability coefficient (ml min^{-1})			Chloride decrease (mM)	
	Before HAc	After HAc	Difference	Before HAc	After HAc
2	0 06	0 11	83	15	32
3	0 09	0 22	144	25	36
4	0 06	0 09	50	21	37
Mean value	0 07	0 14	100	20	35
5	0 09	0 13	44	26	30

can be shown that in such experiments the disappearance rate of acetic acid is less than that for hydrochloric acid whereas in the cat's stomach the reverse is true Note that the volume decreased in the acetic acid instillation experiments because of the hypotonicity of the solution

B Influence of acetic acid on subsequent instillation of hydrochloric acid

Similar experiments as described above were performed in three consecutive steps First 170 mM HCl was introduced and allowed to remain to diffuse for one hour (samples taken every 15 min) See Fig 2A In step two the same procedure was performed with 170 mM HAc and in a third step another 170 mM HCl was instilled

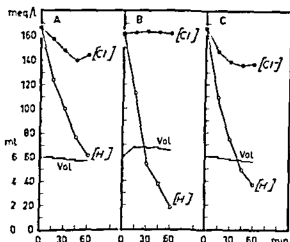


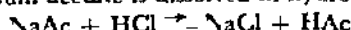
Fig 3 An experiment with consecutive instillations of A 6 ml of 170 mM hydrochloric acid B 6 ml of 170 mM hydrochloric acid to which was added sodium acetate in powder form to a final concentration of 170 mM C 6 ml of 170 mM hydrochloric acid

See Fig 2B It was shown that in the second instillation of hydrochloric acid there was a higher permeability coefficient for hydrogen ions than in the first instillation That this was not the case when the two instillations were not separated by an instillation of acetic acid was shown in experiments where two consecutive hydrochloric instillations were analysed In this case no increase in the permeability coefficient could be obtained On the contrary most of the experiments showed a decrease in the permeability coefficient for the second instillation The results are illustrated in Fig 2 and Table II The permeability coefficient had increased on an average by about 100 per cent At the same time there appeared a significantly lager dip in the chloride concentration curve after the treatment with acetic acid It should also be noted that during the instillation of acetic acid the stomach volume decreased markedly as it did to a lesser extent in the second hydrochloric acid instillation

Macroscopically no apperent increase of mucus formation was obtained

C Effect of a mixture of hydrochloric acid and of sodium acetate on the permeability properties for hydrochloric acid

If sodium acetate is dissolved in hydrochloric acid the reaction



$$NaAc + HCl \rightarrow NaCl + HAc$$

takes place The pH of the solution increases because the pKa of acetic acid is higher than that of hydrochloric acid The situation for this solution will thus be similar to that reported under B Accordingly a persistent increase of the permeability for hydrochloric acid was obtained An example of such an experiment is given in Table II as experiment no 5 and in Fig 3 In this experiment hypertonicity obtained because sodium acetate was added to a final solution of 170 mM caused a volume increase

Discussion

In non secreting cat stomachs it was shown that the acidity decreased more rapidly when acetic acid was introduced than when hydrochloric acid was introduced This confirms earlier results of Teorell (1939) Such an increased disappearance rate could

be due to a possible increase in vascularisation but may also be explained by a rapid permeation of the undissociated weak acid not only through the water phase but also through lipid membranes (Teorell 1959 Hogben *et al* 1957 Schanker *et al* 1957) This explanation has been applied to acetic acid as well as to many other weak acids and weak bases (Bilski and Obrink 1959 Hogben *et al* 1957 Milne Scribner and Crawford 1958 Schanker *et al* 1957)

In this paper it could however be shown that a second instillation of hydrochloric acid made after treatment with acetic acid resulted in a higher permeability coefficient for hydrogen ions than did the first instillation The introduction of acetic acid thus had a persistent effect on the permeability properties of the gastric mucosa This may be compared with the observations of Babkin and his group on the persistent effect on the secretory capabilities of the gastric mucosa after treatment with acetic acid

During the experiments with acetic acid the total acidity usually decreased from 170 mM to an average of 10 mM In this region the degree of dissociation for acetic acid varies between 0 01 and 0 04 Thus most of the acid was undissociated during the whole experiment In the mucosal cells as well as outside of the stomach the pH is much higher (pH around 7) Under these conditions the concentration of the undissociated acetic acid is probably not more than 1 per cent dissociation coefficient equals 0 99 The concentration of the undissociated form of acetic acid on the serosal side of the membrane can consequently be considered as being practically zero The concentration gradient for undissociated acetic acid over the mucosal membrane will thus be directly proportional to the total concentration of acetic acid within the gastric contents

In Fig 1 and Fig 2 the concentration of the hydrogen ions is plotted also on a semilogarithmic scale showing a straight line disappearance rate for the hydrogen ions Thus the disappearance can be treated as a diffusion following the formula

$H = H_0 e^{-kt/p}$

Where H = the actual hydrogen ion concentration or concentration of acid at time t

$H_0 = H$ at time $t = 0$

k = the permeability coefficient (ml/min)

t = time in minutes

p = the introduced volume (ml)

This formula was shown to be valid for hydrochloric acid by Teorell (1933 1947) and Öbrink (1948) It is applicable if the actual concentration of the molecules outside the stomach is so small that it can be considered as being zero The diffusion is looked upon as a molecular one

Our results suggest that the acetic acid also disappears at a rate directly proportional to its concentration within the stomach

The coupling between the probable distribution of the acetate as mentioned above and the observed changes in the permeability properties of the gastric mucosa and its decreased secretory capabilities is however not yet understood One should however consider the probable accumulation of acetate within the mucosal cells in confirmation of Martin (1963) A concomitant secretion in a diffusion experiment would give a falsely low permeability coefficient according to Öbrink and Waller (1964) In the related experiments of type B there was a mean volume increase of 0 1 ml before but a mean decrease of 0 3 ml after the introduction of acetic acid This difference in volume changes could possibly be explained if a hypertonicity develops in the mucosal cells due to intracellular accumulation of acetate A small secretion at the start of the experiment cannot be excluded however and part of the effect of acetic acid could then be to

inhibit that secretion. The permeability coefficient for hydrochloric acid would consequently be estimated as somewhat too low in the first part of the experiment due to the presence of hydrochloric acid secretion.

If the disappearance rate for (H^+) increases more than does the corresponding permeability for sodium ion influx, then chloride has to make a larger dip in the concentration curve than usual because of the requirements for electroneutrality. This did actually happen as can be seen in Fig. 2.

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Acta physiol scand 1964 62 429—435

From the Department of Physiology University of Goteborg Sweden

Renal Vasoconstriction and the 'Defense Reaction'

By

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Abstract

Feigl E B Johansson and B Lofving *Renal vasoconstriction and the defense reaction* Acta physiol scand 1964 62 429—435 — Using the Horsley Clarke technique in cats diencephalic structures were explored with stimulating electrodes Venous outflows from the renal and skeletal muscle vascular beds were measured Intense renal vasoconstriction was induced by hypothalamic stimulation in areas where sympathetic vasodilator fibres were activated It is suggested that renal vasoconstriction is an integral component of the vascular adjustments which occur in the defense reaction

It is generally agreed that the renal vessels are richly supplied with sympathetic nerve fibres Direct peripheral stimulation of these fibres can induce a drastic decrease in kidney blood flow The sympathetic nerve frequency response curve for the renal vessels is somewhat less steep than that for the muscle vessels at lower impulse rates The flow response in the kidney is otherwise similar to that obtained in skeletal muscle when the vasoconstrictor fibres of the two tissues are stimulated at corresponding frequencies (Celander 1954 Khajutin 1962) There is evidence however that during normal resting conditions the tonic vasoconstrictor fibre influence on the renal blood flow is weak or even negligible (Smith 1951 pp 411—419) Moreover when the activity of the bulbar vasomotor centre is enhanced by decreased baroreceptor and/or increased chemoreceptor influence the renal vessels are usually little engaged in the vasoconstrictor response (e g Hartmann Orskov and Rein 1937) They differ in that respect *from intestinal and skeletal muscle vessels (Lofving 1961 a and b Kullip 1963)* It seems therefore that the vasoconstrictor fibres to the kidneys are not greatly activated from the bulbar vasomotor centre in its homeostatic receptor controlled activity except under intense excitation The difference between the renal and the skeletal muscle vasoconstrictor fibres is probably more quantitative than qualitative It has been suggested that the central neuron pools which control the renal vessels exhibit a

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relatively high threshold to excitatory influences from cardiovascular homeostatic reflexes (Folkow Johansson and Löfving 1961) The question arises whether there are patterns of increased sympathetic activity where the renal vessels are more extensively engaged

Löfving (1961 b) observed that hypothalamic stimulation could produce renal vasoconstrictions that were very powerful as compared with the weak or often insignificant effects induced *via* carotid sinus reflexes Renal vasoconstriction in response to hypothalamic stimulation was also reported by Takeuchi *et al* (1960 and 1962) It was thus considered to be of interest to explore the diencephalon systematically in experiments with simultaneous blood flow recordings in kidney and other vascular beds It will be reported here that stimulation of diencephalic structures from which the sympathetic vasodilator fibres to the muscles are activated also produces dramatic renal vasoconstriction The muscle vasodilator area of the diencephalon is thought to be an integration centre for the over all defense reaction (Abrahams Hilton and Zbrozyna 1960)

Methods

Thirty-one cats were used After induction with ether the anesthesia was adjusted to a superficial plane by the intravenous injection of small doses of chloralose (30—50 mg/kg b w) The skull was opened with a dental drill and the head placed in a Horsley Clarke stereotaxic apparatus which was used to position the stimulating electrodes After the experiment the brain was removed and fixed in formaline saline solution Stimulation points were confirmed by tracing the electrode track in fixed sections

Steel monopolar stimulation electrodes were used which were electrolytically sharpened and covered with electrical insulating varnish except at the very tip They were bilaterally introduced for stimulation of corresponding areas of the brain on both sides of the midline The indifferent electrode was inserted into the muscles of the forelimb Rectangular cathodal stimulation pulses were delivered from a Grass model S 4A stimulator The usual stimulation characteristics were a pulse duration of 2 msec between 2 and 3 V to the electrodes in parallel, and a frequency of 70 imp sec though often the frequency was varied between 5—100 imp sec.

The abdomen was opened and the large and small intestines were extirpated To eliminate the cardiovascular effects of concomitantly released catechol amines both adrenal glands were excluded from the circulation by ligating all adrenal veins Care was taken not to damage the sympathetic supply to the kidney Hydrocortisone (5 mg) was given i m to compensate for the loss of corticoid secretion

Heparine was given as an anticoagulant Blood pressure was measured with a mercury manometer connected to a femoral artery Muscle blood flow was measured as the venous outflow from the deep femoral vein the circulation below the ankle being eliminated by a tight ligature This preparation gave a blood flow which was predominantly from the skeletal muscles of the hind limb The renal vein was cannulated and the venous outflow measured The venous outflow from the two regions was directed to transparent drop chambers filled with silicone oil and returned to the animal *via* suitable veins (Lindgren 1958) The drops were counted with a photocell drop counter which activated ordinate writers where the height of the ordinates was proportional to the period between drops (Clementz and Ryberg 1949) The height of the ordinate inscribed was thus inversely proportional to the rate of flow Recording was done on a kymograph.

In 4 cats urine flow was studied together with renal and muscle blood flow A short polyethylene catheter was inserted into the lumen of one of the ureters and the drops of urine coming out were recorded. To insure a relatively high control diuresis these animals were given *100 ml of water* by stomach tube about two hours before the start of the experiments and a continuous infusion of Tyrode solution (0 2—0 5 ml/min) during the experiments

In most experiments aortic and carotid baroreceptor reflexes were eliminated at the beginning or during the course of the experiment by cutting both cervical vagi and both carotid sinus nerves. With this preparation it was possible to study the effects of central nervous system

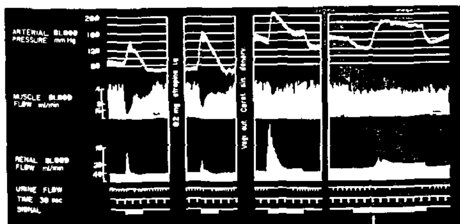


Fig 1 Cat 2 6 kg Chloralose Stimulation of the hypothalamic defense a ea at 60 imp/sec 2 msec and 3 V in panels 1 to 3 In panel 4 the electrodes were lo vered slo ly through the sympatho-inhibito y region of the hypothalamus reaching the defense area at arro The vasodilatation obta ned in skeletal muscle in panel 1 is markedly reduced by atropine (panel 2) and abolished after subsequent elimination of the buffer reflexes (panel 3) The latter p ocedure augments the renal vasoconstriction induced by hypothalamic stimulation and a susta ned reduct on in renal blood flow follows the peak response in panel 3 The vascular changes in the kidney are associated with marked antidiuresis Kidney weight 8 5 g

stimulation with and without secondary baro eceptor reflexes which might be evoked by changes in the pulse or mean blood pressure

To avoid changes in muscle blood flo v due to alterations n skeletal muscle activity resulting from brain stimulation most of the an mals were curarized with Flaxedil (5 mg/kg b w) Artificial respiration was then ma ntained by a pump prev ously adjusted to barely suppress spontaneous respiration

Results

Fig 1 shows a recording of arterial blood pressure muscle and renal blood flo s and urine flow from a representative experiment in which the defense reaction area of the anterior hypothalamus v as stimulated The animal was curarized with Flaxedil The vagal and carotid sinus nerves were intact in the first part of the experiment In the first section of Fig 1 hypothalamic sumulation gave a rise in blood pressure a pronounced increase in muscle blood flow and a renal vasoconstriction accompanied by a reduced urine flow The flow resistance in the skeletal muscle region was reduced to about 15 per cent of control during the initial part of the stimulation period At the same time renal flow resistance increased some 300 per cent at the peak of the response The muscle vasodilation was shown to be due to activation of the cholinergic vasodilator fibres since it was markedly reduced after close arterial administration of atropine to the skeletal muscle region (second panel of Fig 1)

After atropine muscle flow resistance decreased to about 65 per cent of control a marked reduction in the extent of vasodilation as compared to the first section of the figure The induced transient increase in renal flow resistance was of the same order of magnitude as before atropine (note that the rise in blood pressure was greater in

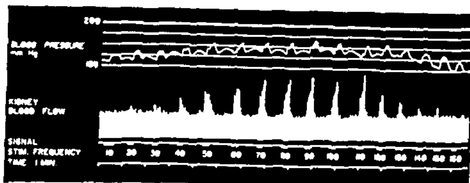


Fig 2 Cat 2 4 kg Chloralose Both adrenals ligated A series of stimulations at a point in the vasodilator area of the hypothalamus using the same voltage and pulse duration but varying the frequency Ordinate height inversely proportional to blood flow

the second part of the figure) The moderate decrease in muscle flow resistance obtained *after atropinization indicates an inhibition of vasoconstrictor fibre activity* to the skeletal muscles during the hypothalamic stimulation Such an inhibition might be induced either directly from the defense reaction area or it could be a secondary effect of *increased baroreceptor discharge in association with the rise in blood pressure* To differentiate between these two possibilities the vagal nerves were cut and the carotid sinus regions were denervated by infiltration with a local anesthetic (2 Xylocain) The third section of Fig 1 shows the response to hypothalamic stimulation with the same stimulation parameters as before but without the buffer reflexes The muscle vasodilatation was then totally abolished muscle flow resistance remaining essentially unchanged while the renal vasoconstrictor response was greatly augmented

Renal blood flow resistance was increased initially to approximately 8 times its prestimulatory value and renal blood flow was reduced from about 4 ml/g of tissue/min to about 0 7 ml/g of tissue/min The intense renal vasoconstriction subsided within a minute in spite of continued stimulation and then leveled out at a renal flow resistance some 70 per cent above control This level of increased resistance was maintained during the remainder of the stimulation period

The last section of the recording in Fig 1 was obtained when the electrodes were slowly moved down through the diencephalon during continuous stimulation There was first a fall in blood pressure associated with a moderate increase in muscle blood flow *a 10—15 per cent reduction in heart rate and no apparent change in venous* outflow from the kidney This response and the position of the electrodes indicated stimulation of the hypothalamic sympatho inhibitory area (Folkow Johansson and Öberg 1959) When the electrodes were lowered slowly and reached the defense reaction area (arrow) the blood pressure increased and a renal vasoconstriction was obtained This latter effect was more gradual in onset due to the more gradual engagement of the defense area in the stimulation of panel 4 as compared to panel 3 The intense peak response was therefore not seen The extent of sustained renal vasoconstriction was however largely the same in the two cases and there was simultaneously a pronounced antidiuresis Urine flow was reduced more than renal blood flow and started again immediately after stimulation

Fig 2 illustrates a frequency response analysis of renal vasoconstriction during stimulation in the vasodilator area of the hypothalamus Voltage and pulse duration were kept constant The figure shows that an essentially maximal response was obtained at a stimulation frequency of 70 imp/sec and that further increase did not enhance the response The frequency response characteristics for eliciting renal vasoconstriction from the hypothalamic vasodilator area appear to be the same as for eliciting active vasodilatation in the muscle The maximal responses were usually obtained with a stimulation frequency of 70 imp/sec and a pulse duration of 2 msec It was not possible to separate the renal and muscle responses by stimulating at different frequencies The relative magnitudes of the two responses were equal as stimulation frequency was varied

Discussion

The diencephalon and those portions of the rostral mesencephalon which can be reached through the anterior cranial fossa were explored Wherever it was possible to evoke active cholinergic vasodilatation in the muscles a concomitant renal vasoconstriction was found The most powerful effects were seen upon activation of the hypothalamic area considered to be the integration centre for the defense reaction (Abrahams Hilton and Zbrozyna 1960) At optimal stimulation frequencies for activation of this area about 70 imp/sec renal blood flow resistance was often increased some 6—8 times during the peak of the response This corresponds roughly to the extent of renal vasoconstriction obtained with direct stimulation of the total renal vasoconstrictor fibre supply at about 10 imp/sec (cf Celander 1954) It should be remembered that the discharge rate of the peripheral sympathetic fibres is far lower than the frequencies used to drive hypothalamic structures controlling the sympathetic fibres often with a reduction factor of 1/10—1/25 (Pitts Larrabee and Bronk 1941) At lower rates of hypothalamic stimulation the extent of the renal vasoconstriction was seen to parallel the muscle blood flow increase and the blood pressure change This is in agreement with the idea that the renal vasoconstriction may be part of the same pattern of sympathetic discharge which engages the sympathetic vasodilator fibres

The marked renal vasoconstriction resulting from hypothalamic stimulation in this study is in strong contrast with the slight or almost insignificant renal vasoconstrictions usually observed when baro or chemoreceptor reflexes are elicited (Löfving 1961 a Killip 1963) Vasoconstriction in the intestine and skin with activation of the muscle vasodilator fibres has been reported to occur upon stimulation of the anterior hypothalamic area (Eliasson *et al* 1951 Lindgren 1955 Abrahams Hilton and Zbrozyna 1960 Uvnäs 1960 Cobbold *et al* 1964) The present experiments indicate that renal vasoconstriction forms an important part of the pattern considered to constitute the defense reaction If this reaction serves the purpose of preparing the animal for fight or flight which will require great muscular exertion it is reasonable that kidney blood flow will be decreased the more so as renal flow may take as much as 25 per cent of the cardiac output at rest If sudden intense muscular activity is to be supported by the cardiovascular system a shortlasting restriction of kidney blood flow is consistent with the over all economy of the organism in an emergency situation

It appears reasonable that the steadily operating homeostatic reflex control of the baro- and chemoreceptors does not interfere significantly with the blood supply to the

ors, in mainly responsible for the maintenance of the milieu interieur except in emergency situations like severe blood loss etc The contrast between the dominating hypothalamic influence and the relatively weak reflex renal vasoconstrictions induced by unloading the baroreceptors or stimulating the chemoreceptors thus appears to be functionally purposeful At the same time it should be stressed that a powerful baroreceptor activation seems to be effective in curtailing the hypothalamically induced renal constriction as judged from panels 1—3 of Fig 1 The intense peak vasoconstriction subsides however even after elimination of the baroreceptors This type of adjustment is also seen with direct stimulation of the renal vasoconstrictor fibres and somewhat resembles the autoregulatory escape of the intestinal resistance vessels (Cobbold et al 1964)

Cholinergic muscle vasodilatation associated with a general defense reaction has been reflexly induced by stimulation of superficial pain fibres in experiments on unanesthetized high decerebrated cats (Abrahams Hilton and Zbrozyna 1960) In anesthetized animals stimulation of such afferents was shown to evoke potentials in the hypothalamic defense reaction area (Abrahams Hilton and Malcolm 1962) These studies and the present findings of renal vasoconstriction in response to the diencephalic stimulation suggest that the reflex constriction of renal vessels which occurs on stimulation of high threshold somatic afferents may be partly mediated through the hypothalamus (Johansson 1962)

The reduction in urine flow which accompanied the renal vasoconstriction was probably due to the circulatory changes in the kidney The fact that the diuresis was restored to control immediately after cessation of the hypothalamic stimulation indicates that hormonal factors were probably not involved in this response On the other hand there did not seem to be any direct relationship between the changes in urine flow and the total renal blood flow Urine flow was often reduced much more than venous outflow This suggests that activation of the renal vasoconstrictor fibres from the hypothalamus produces not only a reduction of total renal blood flow but perhaps also a change in the intrarenal distribution of flow This possibility will be subjected to further study

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Acta physiol scand 1964 62 436—448

From the University Institute for experimental medicine Copenhagen Denmark

The In Vivo Effect of Angiotensin on the Proximal Tubular Reabsorption of Salt in Rat Kidneys

By

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Abstract

Leyssac P P *The in vivo effect of angiotensin on the proximal tubular reabsorption of salt in rat kidney* Acta physiol scand 1964 62 436—448 — The purpose of the investigation presented in this report was to study the effect of angiotensin on the rate of proximal tubular reabsorption of filtrate in mammalian kidneys as evaluated from registration of the occlusion time which is the time required from abruption of the renal circulation until the proximal tubules have completed reabsorption of the luminal filtrate A direct momentary and rapidly reversible inhibitory effect of angiotensin is demonstrated the maximally depressed proximal reabsorption rate corresponding to the lowest rate observed in spontaneous variations of the glomerular filtration The results indicate that a further depression of the glomerular filtration rate below that corresponding to the maximum effect of angiotensin is caused by a reduction in the number of nephrons participating in urine formation It is concluded that angiotensin besides its vasoconstrictive effect has a direct tubular inhibitory action thus providing the two primary effects required for a hormonal regulator of renal blood flow proximal reabsorption rate and thereby of glomerular filtration rate

In a previous paper (Leyssac 1963) direct experimental evidence was presented supporting the suggestion of Bojesen (1954) that the proximal reabsorption of sodium is a T max process (i e independent of the filtered load) which is the main limiting factor to the rate of glomerular filtration Consequently variations in the glomerular filtration rate are merely reflections of primary variations in the rate of proximal reabsorption of sodium or the sodium T max

The fairly close parallelism between renal plasma flow and sodium reabsorption (and hence glomerular filtration rate) observed under a great variety of experimental conditions led to the suggestion by Bojesen that some unknown humeral substance present in the plasma exerting a stimulating effect on the cellular transporting capacity of sodium limits the rate of reabsorption of proximal tubular fluid

The presence in normal kidneys of the pressor enzyme renin originally demonstrated by Tigerstedt and Bergman (1898) and the localization of renin to the juxtaglomerular

apparatus (Bing *et al* 1958 1959 1960 1962 Cook and Pickering 1959 Hartoft and Edelman 1961) has renewed the interest in the still unanswered question whether any physiological role can be attributed to the renin angiotensin system (Gross 1962)

It is well established that sodium and mineralocorticoids influence the amount of renin in the renal cortex and the granularity of the juxtaglomerular cells (Tobian 1960) and increasing evidence is accumulating that the renin angiotensin system plays a considerable role in the regulation of aldosterone secretion (Genest *et al* 1960 Laragh 1960 Davis 1961 Mulrow Ganong and Boryczka 1963 Brown *et al* 1963)

The relationship between the renin angiotensin system sodium and aldosterone the vasoconstrictive effect of angiotensin and the localization of renin to the juxtaglomerular apparatus made it an attractive idea that angiotensin might be responsible for the relationship between renal blood flow and proximal sodium reabsorption

The purpose of the present paper was therefore to study the effect of angiotensin on the proximal tubular reabsorption in vivo by a direct method and it was demonstrated that angiotensin has an immediate depressing effect on the proximal sodium T max by which this rate may be reduced to a certain minimum

Methods

M le albino rats we ghing 250 g were used The animals were allowed free access to food and tap water until the onset of the experiment at which time they were anesthetized with sodium amytal 25 mg fo the first 200 g b w and 2 5 n g for each additional 10 g of b w administered i p and supplemented if necessary during the course of the experiment As a routine procedure 1 0 to 1 5 ml isotonic saline was administered slowly i v before priming with inulin to insure a reasonable urine flow

The general techn que fo measurement of occlusion t mes sampl ng of ureteral urine and blood, and the administration of nulin has been outlined in a p evious paper (Leyssac 1963) Bri fly the left kidney was exposed illuminated and observed th ough a dissecting m croscope (Reichert) as for micropuncture A priming dose of 15 mg of inulin was gi en i v followed by a continuous infusion of 0 15 mg min Two or three specimens of urine were collected by means of a cathete in the left u eter for clearance determinations blood samples f om an indwelling catheter in the right carot d artery being collected before the first urine sampling and immed ately after the measur ment of the occlus on time Fifty unt un ts of heparin w e given n 0 1 ml sal ne at the time of priming with inulin in ord r to p event clotting in the catheter for blood sampling

The occlus on time was measured by observation of the superficial tubular lumina on the k dney surface afte interruptio i of the renal blood circulation and represents the time interval from circulatory arrest until no lumina wer left e until all tubular fluid was reabsorbed. The results of measured occlusion times are expressed n rec p ocal terms under the p esent experimental cond t ons these epresent a b trary units of the rate of proximal flu d reabsorpt on The val d ty of this statement has been discussed in d tail (Leyssac 1963) A sl ght mod fication in cutting off th renal circulation has been introdu ed n the later p rt of this investigation In stead of ligating the renal artery an open lamp was fixed round the aorta proximal to th renal arteries This lamp was closed and released with minimal disturban e to the observed k dney and enal ped cl Furthermore this modification left the renal art ry free fo other purposes e g partial clamping and enabled a measurement of occlusion time and subsequent reestablishment of renal circulat on to be pe formed w thin 30 to 40 sec

For test ng the ffect of angiotensin 25 ng (0 1 μg kg) of syntet c al 5-angiot ns n II am de (CIBA) was given as an v inject on mmediately after the last collecting period for clearance determinations At th ery moment when angiotensin reached the kidney that is as the kidney began to pale th aorta was totally clamped and the occlusion time was noted In one experiment shown th tubules w allowed to efill by opening the clamp and 2 to 3 m n after the first occlus on the aorta was clamped again and the occlusion time noted without giv ng any mo e ang oten in In another experiment the occlus on time was registered m mediately afte the last urine collecting period without prev ous adm nistration of angiotens n

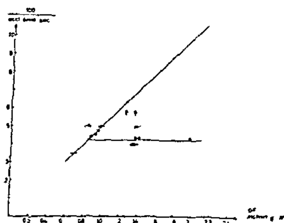


Fig 1 Effect of syntetic angiotensin on the reciprocal occlus on tim e at spontaneous variations in GFR reg istered immediat ly before the ad min stration of angiotens n Hori zontal lines represent the mean value and range of reciprocal occlusion times Oblique regression l ne give the relationship between the two parameters without the administration of angiotensin

The tubules were then allowed to refill angiotensin was given and the occlusion time was registered again

The effect of partial clamping of the renal artery was tested in a group of an mals with a rather tight partial silver clamp (0 20 mm) placed arround the renal artery dissected free from the renal vein. Usually the immediate effect of partial clamp ng was blanch ng of the kidney occlusion of all the tubular lumina and anuria but in 2 to 3 min the peritubular *capillary blood flow was restored and after 10 to 30 min all the lumina were refilled* and appeared well distended Urin flow however did not begin until 30 to 60 min after clamp ng the renal artery and was not steady until ? to 3 hours later Then kidney function was generally stable for a further ? to 3 hours as indicated by constant clearances of inulin When urine flow reached a stable rate collections for clearance determination were started and after the last *collecting period the occlus on time was measured.*

In a parallel group af animals with partially clamped renal arteries urine and plasma was collected for determination of osmolality when the urine flow remained stable below a flow rate of 1 μl/min Usually two urine collections were taken from each animal during the course of the experiment at two different flow rates After the last collecting period occlus on time was *measured These animals were not gi en inulin since it was observed that the concentration* in the urine from clamped kidneys could reach a value of 5 to 10 g inulin/100 ml urine and would thus noticeably influence the urine osmolality

In another series of experiments instead of clamping the aorta for determination of occl usion time the kidneys were snap-frozen either in a freezing mixture of acetone and sol d carbon dioxide at — 65 C or in the later experiments in isopentane cooled to — 160 C with liquid nitrogen In the control group kidneys were snap-frozen immediately after the last collecting period for clearance determinations in the angiotensin treated group 25 ng of synthetic angiotensin was injected and the kidneys snap-frozen the instant when angiotensin reached the kidneys partially clamped kidneys were snap-frozen when the urine flow was stable below 1 μl/min Snap-frozen tissues were transferred to absolute ethanol at — 20 C and left at this temperature for 6 to 12 days (freeze substitution) After freeze substitution tissues were imbedded in paraffin secti ned and stained as described previously (Leyssac 1963) Internal diameters of proximal tubules were measured in 50 transversially cut, circular super ficial, proximal tubules in each kidney by means of a micrometer ocular

Inulin analyses were performed by the method of Bojesen (1952) modified for microanalysis

Freezing point depression of plasma and urine was determined microcryoscop cally by the method of Ramsay and Brown (1955) using samples 1 × 10 to 1 × 10 ml in volum e collected in micropipettes (Pyrex) under mineral oil Determinations were rounded off to nearest 0 01 C. The instrument was calibrated with known molal solutions of sodium chloride using data from the International Critical Tables (1928) and results are expressed in terms of osmolality

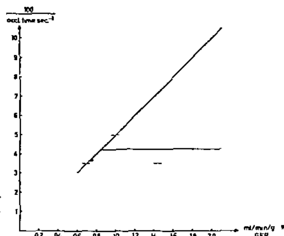


Fig 2 Reciprocal occlusion time at varying GFR in partially clamped kidneys Oblique regression line represents the relationship in spontaneous variations of the two parameters Horizontal lines give the mean value and the range of reciprocal occlusion times after administration of angiotensin

Results

Fig 1 shows the immediate effect of 25 ng of exogenous angiotensin on the occlusion time at spontaneously different rates of glomerular filtration registered immediately before the injection of angiotensin Regardless of the initial occlusion time as expected from the measured glomerular filtration rate before the injection (oblique regression line Leyssac 1963) the effect of angiotensin is an instantaneous depression of the rate of disappearance of proximal fluid to a minimum corresponding to an occlusion time of about 25 sec This effect is rapidly reversible In one experiment (right unfilled circle) the occlusion time of 17 sec before the administration of angiotensin is in the area expected from the measured rate of filtration (1 36 ml/min/g KW) A subsequent injection of angiotensin a few minutes after reestablishment of the circulation changed the occlusion time down to 27 sec (arrow pointing downwards) In another experiment in which a filtration rate of 1 27 ml/min/g KW was observed an occlusion time of 22 sec was registered after angiotensin a combination of measurements which is significantly different from that expected without giving angiotensin A few minutes after reestablishment of the circulation and refilling of the tubular lumina — without giving more angiotensin — the occlusion time had returned to 17 sec (arrow to the left) a value to be expected from the filtration rate measured before the administration of angiotensin

Since there are reasons to assume that a kidney with its renal artery partially clamped has a release of endogenous angiotensin exceeding that of normal kidneys it was tempting to examine whether such arterially clamped kidneys would show the same maximal inhibition of the rate of proximal reabsorption as that demonstrated after exogenous angiotensin

Fig 2 shows occlusion times at different rates of glomerular filtration in partially clamped kidneys Below a filtration rate of about 0 8 ml/min/g KW the occlusion time has reached a maximum only slightly above that obtained with synthetic angiotensin Filtration rates are depressed far more than can be explained on the basis of the

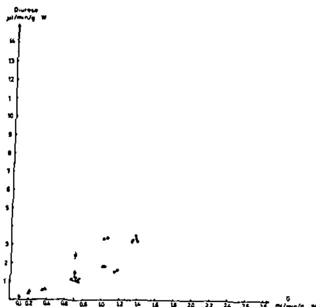


Fig 3 Diuresis at varying GFR from control and partially clamped kidneys Note that b lo a GFR of 0 7/ml/min/g KW the diuresis falls in linear proport on to GFR ● Cont ol k dney ▲ Clamped Kidney

depressed rate of reabsorption which can be responsible only for a depression of the glomerular filtration rate to a magnitude of 0 6 to 0 7 ml/min/g KW

In Fig 3 the diuresis is plotted as a function of the glomerular filtration rate Above a filtration rate of 0 7 ml/min/g KW no correlation exists between the two parameters but below a filtration rate of 0 7 ml/min/g KW the rate of urine flow falls from a value of 1 μl/min/g KW proportionally to the reduction in the filtration rate a fact which is interpreted to indicate that reductions in the rate of filtration below a value of 0 7 ml/min/g KW are due to a decreasing number of tubules taking part in urine formation

The histological picture of a rat kidney in which a spontaneous glomerular filtration rate of 1 65 ml/min/g KW was registered immediately before the kidney was snap frozen is shown in Fig 4 All the tubules are found to be wide open and circular in the functioning state and they were so throughout the entire section

The histological picture of a rat kidney in which the spontaneous filtration rate was found to be 0 5 ml/min/g KW in the collection period immediately before snap-freezing is shown in Fig 5 It is seen that among the open functioning tubules one may find single or groups of proximal tubules occluded This is the histological evidence for the statement that below a glomerular filtration rate of 0 7 ml/min/g KW a further fall in filtration rate is due to a decreasing number of nephrons taking part in urine formation when the occlusion progresses to an increasing part of the nephron population

Thus urine flow rates below 1 μl/min/g KW from rats under the present experimental conditions indicate that the glomerular filtration rates are reduced below 0 7

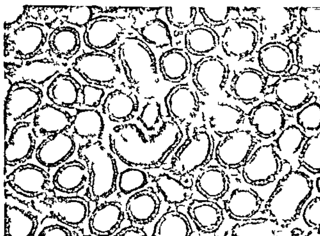


Fig 4 Snap-frozen rat kidney GFR 1 65 ml/min/g KW All tubules a e wide open and circular Magnific × 225 (Thickness of histological section 3 μ)

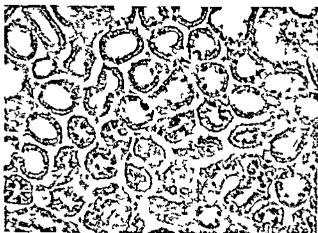


Fig 5 Snap-frozen rat kidn y GFR 0 5 ml/m n/g KW Note the totally and part ally occluded p o imal tubules among the still open and ci cular lum na of func t on ng tubules Magnific × 225 (Th ckness of h stolog cal sect on 3 μ)

ml/min/g KW due to the occlusion of major or minor parts of the nephrons population

The osmolality of plasma and urine from partially clamped kidneys was measured at various urine flow rates below 1 μl/min/g KW (Fig 6)

Occlusion times in this group of animals were equal to those obtained in partially clamped kidneys having filtration rates below 0 7 to 0 8 ml min/g KW (Fig 2) Urine osmolality decreases with decreasing flow rates approaching isosmolality indicating that the concentrating capacity is reduced when the renal function is so much impaired that a decrease in glomerular filtration rate is caused by a decrease in the number of functioning tubules The decrease in urine osmolality with decreasing urine flow rates below 1 μl/min/g KW shows that changes in the secretion of antidiuretic hormone cannot have been responsible for the reduction in water excretion.

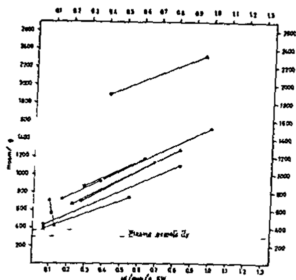


Fig 6 Urine osmolality (ordinate) at varying rates of urine flow (abscissa) from partial clamped kidneys The symbols represent different animals and lines are drawn between two readings at different urine flows from the same kidney at two periods with an interval of one or more hours Both periods were collected after clipping the kidney The broken lines give the range of osmolality from plasma samples

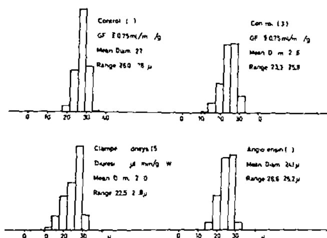


Fig 7 Distribution of internal diameters of proximal tubules at spontaneous variations of GFR (control groups) after angiotensin and in partially clamped kidneys The number in brackets give the number of kidneys examined in each group The diameter of 50 proximal tubules were measured in each kidney Note the skew to the left in the lower three groups

Since the volume of fluid present in the functioning proximal tubules would influence the occlusion time and since it could not be excluded that a difference might exist in the different groups of kidneys examined the internal diameters of transversely cut circular proximal tubules from the outer cortex were measured in each of the four groups investigated The distribution of these internal diameters is shown in the histograms of Fig 7 Although the mean diameter in the control group with filtration rates

above 0 75 ml/min/g KW is somewhat greater than in the other three groups the reduced mean diameters in these groups are seen to be due to a skew distribution leaving the major part of the nephron population with diameters equal in all four groups In such cases a greater scatter in the occlusion times of the tubules is to be expected However the measured occlusion time was always that of the major part of the tubules (completing the occlusion within a period of 1—2 sec) consequently differences in the internal diameters did not affect the measured occlusion times

Discussion

The fundamental concept outlined by the school of Homer Smith (Smith 1951) that glomerular filtration rate is determined by the filtration pressure (glomerular factors) only with the idea of a load-dependent proximal reabsorption was based upon the presumption of a constant pressure in the space of Bowman s capsule

Direct measurements in recent years do not however fit easily into this general interpretation The demonstration by Wirz (1955 1956) and by Gottschalk and Mylle (1956) that the proximal intratubular hydrostatic pressure (and hence the pressure in the space of Bowman) is not constant infer that changes in the intrarenal pressure must significantly influence the rate of filtration (Wirz 1956) It was not realized except by Brandt Rehberg (1929) and by Bojesen (1954 1957) that this might also be true for such changes in the pressure which might be caused by primary changes in the rate of reabsorption

Direct measurements on proximal tubular fluid (Walker *et al* 1941 Windhager and Giebisch 1961) have demonstrated that a concentration gradient for sodium across the proximal tubular wall does not normally exist and thus can not explain the load dependency of the proximal reabsorption as postulated by Smith (Smith 1951 p 376—378)

To explain the so-called glomerulo-tubular balance without this claim of a rate limiting concentration gradient but still satisfying the conventional idea of a load dependent proximal reabsorption Gertz (1963) makes the assumption in a micropuncture study in rats on the transtubular sodium chloride flux that during reductions of the proximal luminal diameter the flux keeps constant per unit surface area which area is supposed to diminish Thus any reduction in the filtration rate would cause a reduced luminal diameter and therewith reduce the proximal rate of reabsorption However I (Leyssac 1963) have shown that the proximal reabsorption continued at an unaltered rate with decreasing internal diameter until all the luminal fluid was reabsorbed following ligation of the renal artery and the present demonstration of unchanged internal diameters of the major part of the nephron population within a great range of filtration rates is incompatible with the idea of changes in the luminal diameter as a function of filtration rate Also the demonstration of unchanged hydrostatic pressures in these proximal tubules of clamped kidneys with reduced filtration pressure and very low filtration rates (Leyssac 1964) speaks against this idea This does not of course invalidate the idea of a constant flux per unit surface area since changes in the luminal diameter should by no means affect the enormous area of the brush border surface of the proximal tubules The relevance of this physical fact was rejected by Gertz only because it would imply according to the traditional way of thinking that at reduced filtration rates the lumen would be emptied which actually is not the case in the low range of filtration rates observed spontaneously

Direct experimental evidence has finally been presented (Leyssac 1963) that the proximal reabsorption of salt is a T max process which always operates at the T max i. e. independent of the "filtered load" and is the limiting factor in the rate of glomerular filtration. As a consequence it must be stated, as emphazised by Bojesen (1954) that it is impossible from clearance studies to answer the question whether observed changes in the rate of excretion of water and predominant electrolytes are caused by changes in the filtration pressure (glomerular factors) or by tubular factors since the rate of excretion, which is within the error of the clearance method, is determined not only by the effective filtration pressure and the overall rate of reabsorption, but also by the distribution between proximal and distal reabsorption and by the hydrodynamic resistances to flow through the nephron (i. e. the luminal diameter).

This implies that none of previous investigations using indirect methods could possibly establish any tubular effect of angiotensin nor could such an effect be excluded. It was therefore necessary to approach the question of a primary effect of angiotensin on the proximal reabsorption of sodium by a more direct method. This was first tried by Leyssac, Lassen and Thaysen (1961) using the kidney cortex slice technique of Lassen and Thaysen (1961).

Further investigation with this method using thin slices were negative but several types of evidence were presented substantiating the view that at least the major fraction of the sodium is lost from the incubated slices exclusively by diffusion (Bojesen and Leyssac, in prep.). Consequently any effect of angiotensin using this crude technique could not be expected. A more refined analysis using a more well-defined geometry of the tissue did, however, disclose a small fraction of the sodium pool (about 10—20 %) which is not in diffusion equilibrium with that of the medium and may be subject to active transport (Bojesen, Leyssac and Svejgaard Nielsen, in prep.).

Gertz (1962) tried the effect of angiotensin on sodium flux by microinjections of angiotensin into the lumen of rat proximal tubules. An inhibition of about 30 % was observed with concentrations not less than 10 M (equal to about 10 mg per 100 ml). However, it does seem more likely that angiotensin should act from the peritubular surface (blood side) in a similar way as vasopressin, another octapeptide, seems to do on the water flux in the distal part of the nephron.

A direct demonstration of a tubular effect of angiotensin independent of the known vascular effect by an administration into systemic or renal circulation with persistant renal blood flow *(flowing system)* was *a priori impossible*, *since any tubular effect* would not be likely to manifest itself before a reduction in the renal blood flow caused by the vasoconstrictive effect. Since furthermore reductions in the filtration pressure seem to be a stimulus to the release of endogenous angiotensin somehow (see Leyssac 1964), it should be predicted that in a flowing system any pressor substance causing reduced renal blood flow would cause a reduction in the rate of proximal reabsorption, not by a direct tubular effect, but via the release of endogenous angiotensin.

A direct approach using the occlusion time method seemed possible, since a sudden interruption of the renal circulation and filtration, upon which the method is based, does not influence the rate of reabsorption eo ipso. Therefore, the immediate vascular effect of exogenous angiotensin, in principle equal to the interruption of circulation, does not itself *exert* an effect on the rate of reabsorption, and recent experiments have shown that noradrenaline in weight doses 25 times that of angiotensin (600 ng) has no direct effect at all on the occlusion time (Leyssac, in prep.). If, however, measurements of occlusion times are *to* give any information about changes in the rate of reabsorption

from one experiment or group of experiments to the other all other factors determining the rate of disappearance of proximal luminal fluid must be kept constant This seems to hold true for the present experiments since the composition of the ultra filtrate has been kept constant and since it is excluded that differences in the internal diameters in the groups investigated have influenced the observed occlusion time

Accordingly the demonstrated effect of angiotensin on the occlusion time under the present conditions means that the maximum effect of exogenous angiotensin is a primary inhibition of the proximal reabsorption rate to a minimum value giving a filtration rate of about 0 7 ml/min/g KW This was exactly the minimum glomerular filtration rate found in spontaneous variations in the control group (Leyssac 1963) It is emphazised that an effect of exogenous angiotensin is only demonstrable with high rates of filtration since at the low filtration rates the rate of reabsorption is already inhibited to its minimum most probably due to the effect of endogenous angiotensin as indicated by the conclusions drawn from the present investigation

These experiments therefore show that with a given filtration pressure the filtration rate may show variations from about 2 2 to 0 7 ml/min/g KW exclusively due to variation in proximal tubular reabsorption of fluid (or T max of salt) and this physiological variation may be explained solely by variation in the release of angiotensin

This of course does not imply that changes in the filtration pressure does not influence the rate of filtration at all It only means that the predominant determinant is the rate of reabsorption which determines whether the filtration is maintained at a rate of 0 7 1 0 or 2 0 ml/min/g KW Alterations in the filtration pressure when they occur may change this rate moderately e g in dilution diuresis and other mechanical diuresis Although this change except under extreme experimental conditions is within the error of the clearance method it may significantly influence the rate of water and electrolyte excretion (Bojesen 1954)

The finding of a close relationship between glomerular filtration rate and the length of the proximal tubules (Hanssen 1961) are consistent with both the previous and the present findings At a certain salt T max determined most likely by the rate of release of angiotensin the total volume reabsorbed per min in a long proximal convolution will be greater than in a short one As a secondary consequence the rate of filtration will be greater in the nephron with a longer proximal tubule

In partially clamped kidneys the occlusion time has reached a maximum only slightly above that obtained with synthetic angiotensin no matter how much the rate of filtration was reduced Thus depression of the rate of proximal reabsorption in clamped kidneys would only depress the filtration rate to a value of about 0 7 ml/min/g KW The further reduction observed in the rate of filtration is simply explained by the decrease in filtration pressure caused by the arterial clamp No matter how much angiotensin is released the rate of reabsorption cannot be inhibited any more Consequently any reduction in filtration pressure will permit less fluid to be filtered but the same volume as before will necessarily be reabsorbed per min due to the T max character of the proximal reabsorption Accordingly a smaller fraction of the filtered volume will reach the distal part of the nephron the urine flow decreases and with still further reductions in filtration pressure less can be filtered than can be reabsorbed The proximal tubule therefore occludes The decreasing number of functioning tubules will consequently reduce the measured rate of overall filtration The demonstration of a linear correlation between the diuresis and glomerular filtration rate below 0 7 ml/min g KW and the histological evidence of occluded proximal tubules

below this rate of filtration strongly support this interpretation which is furthermore supported by the demonstration in rabbits (Kruhoffer 1950) that glucose T max was reduced with reductions in the filtration rate below about 10 ml/min whereas no changes in glucose T max were observed above this level

As a consequence to the proximal reabsorption of an increasing fraction of the filtered volume the concentration of inulin in the late proximal fluid will increase considerably This is in accordance with the observation of very high concentrations of inulin in the final urine from these partially clamped kidneys in which the concentrating capacity is shown to be reduced This finding is well known clinically in cases of stenosis of the renal artery Occlusion times in the same range of values in equally clamped kidneys not having inulin indicates that a possible osmotic effect of inulin has played an insignificant role only Even a slight osmotic effect of inulin does not, however invalidate the conclusion that the rate of reabsorption in partially clamped kidneys is depressed to a certain minimum since the occlusion time showed no tendency to a further increase no matter how much filtration rates were reduced If the release of angiotensin from such clamped kidneys can be shown directly to be actually increased to values equal to or surpassing that which would effect the maximum tubular inhibition this would account for the depression of the rate of reabsorption observed in the present experiments

The reduced concentrating capacity observed with decreasing filtration rates in partially clamped kidneys may be caused by several factors concerning which the present data do not permit a differential analysis

The vasoconstrictive effect of angiotensin influences renal blood flow and this study demonstrates in addition a direct action on proximal tubular function which changes the rate of reabsorption within the physiological range of variations The anatomical localization of the renin angiotensin system at the vascular pole of the glomerulus *and the demonstrated tubular effect strongly suggests that angiotensin exerts* its vascular action also in the same anatomical area thereby justifying the conclusion that the renin angiotensin system is a physiological regulator of renal blood flow proximal reabsorption and thereby of the glomerular filtration rate

The final *proof that these parameters* really do vary as a consequence to variations in the release of angiotensin awaits as analysis which would make it possible to measure such minute physiological variations of angiotensin in the renal venous or capillary blood

The data presented in the previous (Leyssac 1963) and the present paper leave no room for the generally accepted interpretation of renal function based upon the fundamental ideas of the school of Homer Smith The acceptance of a rapidly changeable regulated proximal salt T max determining the rate of filtration in contrast to the theory of a constant pressure in the space of Bowman s capsule which was thought not to influence the filtration rate seems to give a more acceptable clue to the understanding of kidney function, predominantly in respect to the important problems of regulation of salt and water excretion and the actions of mineralo-corticoid hormones and diuretics

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The Effect of Partial Clamping of the Renal Artery on Pressures in the Proximal and Distal Tubules and Peritubular Capillaries of the Rat Kidney

By

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Abstract

Leyssac P P *The effect of partial clamping of the renal artery on pressures in the proximal and distal tubules and peritubular capillaries of the rat kidney* Acta physiol scand 1964 62 449—456 — The purpose of this study was to measure the intratubular and peritubular capillary hydrostatic pressures in acute experiments of partial clamping of the renal artery to such a degree that a significant reduction of the filtration pressure was ascertained The renal artery of rats weighing about 250 grams were clipped with a 0 20 mm silver clamp which reduced the urine flow and the glomerular filtration rate to below the range of spontaneous variations and caused occlusion of minor or major parts of the nephron population Intratubular and peritubular capillary pressures were measured directly by the micropuncture technique The results demonstrated that proximal intratubular as well as peritubular capillary pressures in partially clamped kidneys were not measurably different from those of normal control kidneys (12—13 mm Hg) whereas distal intratubular pressures were significantly reduced (from 6 7 mm Hg in normal to 3 9 mm Hg) in clamped kidneys The results are discussed in relation to previous and recent findings and interpretations The hypothesis is proposed that the physiological function of the juxtaglomerular apparatus is to serve as a feed back mechanism whereby changes being a function of the proximal intratubular pressure via the release of angiotensin adjusts the proximal reabsorption and the intrarenal vascular resistance in such a way that proximal intratubular hydrostatic pressures are maintained

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It has been generally accepted that the proximal intratubular hydrostatic pressure normally is determined by the interstitial or peritubular capillary pressure in accordance with the ideas of Homer Smith (Smith 1951) The micropuncture studies of these pressures in rat kidneys by Wirz (1956) and by Gottschalk and Mylle (1956 1957) appear to fit these ideas since it has been well established that the proximal intratubular and the peritubular capillary pressures were always identical under normal conditions as well as during conditions of osmotic diuresis elevated ureteral and renal venous pressure and of antidiuresis These findings further evidenced that an induced increase in the luminal pressure would be transferred to the interstitial

space and vice versa but did not disclose which one of these pressures determined the other under physiological conditions

In the preceding study (Leyssac 1964) it was observed in kidneys with renal arteries partially clamped to such a degree as to significantly reduce the glomerular capillary pressures that the proximal lumina looked well distended and the renal capsule appeared normally tense or at least nearly so Furthermore it was demonstrated that the internal diameters of the major part of the proximal tubules were equal to those of normal kidneys These observations which suggested that the intratubular hydrostatic pressure was not seriously decreased were unexpected in view of the conventional interpretation The purpose of the study presented in this report was therefore to measure the pressures directly in partially clamped kidneys using the micropuncture technique

Methods

Male albino rats weighing ?50 g were used The animals were allowed free access to food and tap water until the start of the experiment at which time they were anesthetized with sodium amytal 25 mg for the first 200 g b w with 2 5 mg for each additional 10 g of b w administered i p and supplemented during the course of the experiment when necessary One to 1 5 ml isotonic saline was slowly administered i v 60 to 90 min before the measurements in order to secure reasonable urine flow

The general set up and technique for micropuncture and intratubular pressure recording as described by Gottschalk and Mylle (1956) has been followed Briefly the left kidney was exposed immobilized and a portion on the surface bathed in Ringer's solution or isotonic saline was illuminated with a Knisely quarts rod apparatus A heating filter was interposed between the light source and the rod The left ureter was cannulated in its upper one third with a polyethylene catheter the tip of which was placed into or very close to the renal pelvis

To measure intratubular or peritubular capillary pressures a Pyrex micro pipette (6—10 μ outer diameter at its tip) was filled with a concentrated aqueous solution of nigrosin The pipette clamped in a de Fonbrune micromanipulator reciever was connected by air filled polyethylene tubing to an adjustable mercury leveling bulb and the manometer The microtip was manipulated into the fluid on the kidney surface using a binocular stereomicroscope (Reichert) at 100—150 × magnification and its capillarity was determined and susbequently checked before each measurement The pipette was manipulated into the lumen of a surface tubule or capillary and the pressure was determined as the pressure which had to be applied to the pipette in order to keep dye from leaving the pipette and tubular fluid from entering it minus the capillarity of the pipette At least two successive pressures were determined in each lumen and the readings were discarded if the pressures differed by more than two mm Hg The manometer was a Tybjerg Hansen condenser manometer connected to a high frequency transducer device and output amplifier (Kaiser) (Tybjerg Hansen 1949)

Pressures were measured in 9 control animals and in 13 animals with a rather tight partial silver clamp (0 20 mm) placed round the renal artery which was dissected free from the renal vein Pressures from clamped kidneys were usually recorded 2 or 3 hours after clamping when urine flow rates had reached a stable level below 1 μl/min In a few cases the diuresis increased during the punctures

Microphotographs of 60 × magnification were taken through the one ocular which was connected to a Robot camera (automatic transport) A Braun electronic flash device attached to a conical lucite rod coated with aluminium foil secured sufficient light for exposures in rapid succession The length of the lucite rod was 300 mm the one polished end had a diameter of 50 mm and the diameter of the other end in contact with the kidney surface was 3 mm An AGFA negative color film was used and exposures were taken at 1/250 sec

Since no measurable pressure drop exists throughout the proximal tubules (Gottschalk and Mylle 1956) the distance of the site of puncture from the glomerulus was not determined in the proximal tubules That the pipette tip was localized in a proximal convolution was ascertained either from the appearance of injected nigrosin in the corresponding distal tubule at later time (within 60 sec in normal kidneys and within 90—180 sec in clamped kidneys)

TABLE I Pressure recordings by Gotts halk and Mylle from normal kidneys from the present control group and from kidneys with a partial obstruction of the renal a tery

Pressures are given as mean value ± standard deviation Number n brackets give the number of puncture

	Proximal tubules (mm Hg)	Small peritubular capillaries (mm Hg)	Distal tubule (mm Hg)	Diuresis (μl/min)
Gottschalk & Mylle 1956	*13 5* ± *2 4* Range 7—21 (193)	*14 0*	—	—
Gottschalk & Mylle 1957	*12 5* ± *2 2* Range 9—20 (50)	—	*6 7* ± *1 6* Range 4 0—12 0 (41)	0 3—7 3
Controls	*12 9* ± *2 3* Range 8—18 5 (46)	*12 9* ± *3 5* Range 9 5—21 5 (13)	—	0 6—10 0
Clamped kidneys	*12 ?* ± *1 9* Range 8—18 2 (40)	*12 6* ± *2 7* Range 8 2—1/ 5 (20)	*3 9* ± *1 2* Range 2 5—6 5 (10)	0 1—1 8

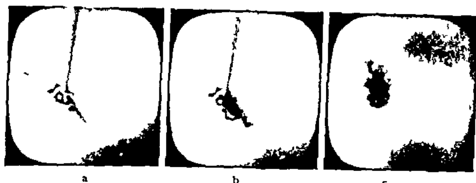
or if no dye appeared by microdissection The s te of puncture in distal tubules were determined by mic odissection The local zat on of the puncture site by microdissection was carried out as described by Gottschalk and Myle (1956) mod fied only n that the kidneys were macerated in concentrated hydrochloric acid d luted 6 10 for 90 min at 37 C

Results

From the table it is seen that the pressures recorded in the present control group are identical with those registered by Gottschalk under similar experimental conditions In the partially clamped kidneys pressures in the proximal tubules and in the peri tubular capillaries are equal to those in the control group but the pressures in the distal tubules are significantly reduced (t = 3 5) from average 6 7 mm Hg in normal kidneys to average 3 9 mm Hg in the clamped kidneys

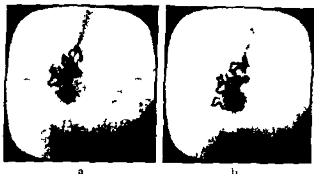
When a proximal tubule in a control kidney was punctured and a microinfusion of nigrosin coloured saline was started the black stain became visible very quickly in the whole proximal convolution distal to the site of puncture (Fig 1a) 40—50 sec from the start of the infusion into the proximal tubule the stained fluid appeared in the distal convolution at the kidney surface (Fig 1 b) In the following picture (Fig 1 c) the micropipette was removed The dye was washed away from the proximal con volution leaving only the distal tubule coloured[1] It is emphazised that the luminal

[1] The present experiments we e not des gned for a quant tative est m t on of l m nal flow rates as ha been performed by others us ng a s m lar t [illegible] most recently by St n hausen (19f3)



a b c

Fig 1 Microphotographs from a control kidney surface during micropuncture
a) Shows the proximal tubule during microinfusion b) Shows the appearance of the distal convolution at the surface 40 sec after the start of infusion c) Shows the persistance of color in the distal tubule after removal of the micropipette



a b

Fig 2 Microphotographs from a clamped kidney surface during micropuncture
a) shows the proximal tubule during microinfusion
b) shows the appearance of the distal convolution at the surface 60 sec after the start of the infusion Note the reduced caliber of the distal convolution

diameter of the distal tubule does not appear significantly different from the proximal luminal diameter However a slightly smaller distal diameter was observed in many nephrons of control kidneys

When a microinfusion of dye was performed into a proximal tubule of a partially clamped kidney the proximal diameter did not appear different from that of the control kidney as shown by the microphotographs in Fig 2 a However not until 60—90 sec after the initiation of the infusion and often much later did the dye in the distal tubule appear at the surface indicating a much reduced luminal flow rate in the clamped kidneys (Fig 2b) From this figure it is quite evident that the luminal diameter of the distal convolution is considerably reduced in comparison with that in the control kidney Similar reductions in luminal diameters were never observed in control kidneys

Discussion

The relative constancy of the proximal intratubular hydrostatic pressure equal to the interstitial pressure observed under different rates of filtration and urine flow apparently has been a main argument both in favour of the concept that the luminal pressure is determined by the interstitial pressure (Wirz 1956) and of the general concept that

filtration rate is insignificantly influenced by such minor changes in this pressure which might be induced by changes in the rate of proximal reabsorption This argumentation seemingly supported the idea of a load dependent proximal reabsorption of sodium an idea against which rather significant evidence ha been presented (Leyssac 1963)

In a discussion of the idea that the interstitial pressure determines the intratubular pressure Swann claimed that the functional distension of the kidney originates from the vascular blood and a large interstitial space (Swann *et al* 1958) Swann believed that the interstitial compartment was ballooned out by plasma and reabsorbate at the instant of clamping the renal pedicle This concept has been seriously criticized by Hanssen (Hanssen 1960)

The present data allow serious doubt to be raised to the general concept of a determinant role of the interstitial pressure to the pressure inside the proximal tubules under normal conditions and would seem quite incompatible with the hypothesis of the kidney as an inflatable balloon proposed by Swann (1960 1961) Provided the peritubular or interstitial pressure determines the proximal luminal pressure it would be expected that the peritubular capillary pressure and hence the proximal intratubular pressure would be significantly lower than normal after clamping the renal artery to such a degree that the filtration pressure is significantly lowered Such reduction in filtration pressure was indicated in the present clipped kidneys by occlusion of groups of the proximal tubules and filtration rates below 0 7 ml min g KW (Leyssac 1964) The present data would indicate that the proximal intratubular and the interstitial pressures in such clipped kidneys are identical with the pressures in control kidneys

It may be argued that the interstitial pressure is maintained due to an increased resistance to venous outflow under this condition However any compression of the thin walled arcuate veins is a most unlikely consequence of a partial arterial obstruction A venous constriction elicited by angiotensin is not likely either since it has been demonstrated by Folkow Johansson and Mellander (1961) that angiotensin is a typical arteriolar or plain muscle cell constrictor with no or insignificant effect on the post arteriolar section of the vascular system and since the sinusoidal cushions described by Swann and co-workers (see Swann 1960) near the junction of the arcuate and interlobular veins almost completely lack smooth muscle cells A significant pressure drop was found across this effluent constriction which was thought to maintain the venous pressure peripheral to the constriction equal to or slightly above the interstitial pressure

The opposite view that the interstitial pressure is a passive reflection of the proximal luminal pressure is apparently more likely in the light of the present experiments The fact that in snap-frozen kidneys the intertubular space appears as extremely thin walled peritubular capillaries the endothelium of which is like a mere cytoplasmatic film located in more or less narrow angles between the tubules (Leyssac 1964 Fig 4) support this interpretation

The maintenance of unchanged proximal intratubular pressures in kidneys partially clamped to such a degree that filtration pressure is significantly reduced seem to indicate that the pressure is maintained by a very efficient regulatory mechanism Provided that partially clamped kidneys have an increased release of angiotensin consistent with the observed low rates of reabsorption in such kidneys (Leyssac 1964) this increased release might obviously be involved in such a regulation

The renin angiotensin system is localized to the juxtaglomerular apparatus (Bing and Kazimierczak 1962) which is the specialized group of cells at the glomerular vascular pole consisting of cells from the distal tubule ("macula densa) from the afferent arteriole ("juxtaglomerular cells) and of a group of cells named Goormaghtigh s cells The Goormaghtigh cell-group is wedged at the vascular pole between the afferent arteriole the efferent arteriole and the macula densa (Faarup 1964 in press) The macula densa of the distal tubule usually *joins and adheres closely to the very* last part of its affiliated afferent arteriole immediately before the arteriole splits into the glomerular capillary tufts At this point the arteriole has lost its muscular media, the plain muscle cells being replaced by the epitheloid cells ("juxtaglomerular cells)

It seems evident that the first point of action of angiotensin in the kidney is distal to the afferent arteriole It is hardly likely that recirculated angiotensin — after dilu tion by blood from other areas and exposure to plasma angiotensinase — should be able to exert a comparable effect on the afferent arterioles

Then angiotensin might affect the glomerular capillaries and/or the efferent arteriole These two possibilities would have direct opposing effects on the filtration pressure a capillary constriction would reduce filtration pressure whereas an effect solely on the efferent arteriole would increase the pressure Although evidence has been presented as mentioned previously that angiotensin is a typical plain muscle cell constrictor with no or insignificant effect on the post arteriolar vascular section and therefore a priori probably not includes any effect on the glomerular capillaries it might be postulated *that these capillaries are specialized and different from other capillaries* The possi bility of an effect including the glomerular tufts may however seem less likely from the following argumentation.

Evidence has been presented (Leyssac 1964) that the filtration pressure is signi ficantly reduced in the glomerular capillaries of partially clamped kidneys The pressure at the very end of the afferent arteriole therefore is hardly unchanged but more likely somewhat lowered If angiotensin had caused a constriction of the glome rular capillaries and the filtration pressure had consequently been further reduced the maintenance of an unchanged proximal intratubular pressure is hardly under standable even though the proximal reabsorption rate was significantly depressed If however the vascular effect of angiotensin is mainly or exclusively on the efferent *arteriole* this fact would tend to keep up filtration pressure thereby supporting the inhibition of the proximal reabsorption in maintaining an unchanged intratubular pressure. The latter possibility obviously seem to be the most reasonable interpretation of the experimental data considered here

The present data *thus support the idea that the physiological actions of angiotensin* serve the regulation of the proximal intratubular hydrostatic pressure However the adequate stimulus to the release of angiotensin is so far unknown in spite of the great *interest* in this question, which was evoked by the evidence of a possible role of the renin angiotensin system in the pathogenesis of renal hypertension and in the regula tion of aldosterone secretion. The evidence presented of a physiological function *in* regulating renal blood flow and proximal rate of reabsorption has furthermore focused the interest on the mechanism by which the release of angiotensin is mediated.

In previous hypothesis *the natu e of the* stimulus to the release of angiotensin has been suggested as either mechanical (pressure) (e g Sellwood and Verney 1954—55 Tobian 1960 Schmid 1962) or chemical (osmotic pressure or sodium concentration) (e. g Maunsbach and Latta 1962 Stamey 1963 Thurau 1963)

These differing previous concepts have the one feature in common that the adequate stimulus to the release of angiotensin is provoked under conditions of changes in the filtration pressure. Evidence for angiotensin release under such conditions has quite recently been given by Skinner, McCubbin and Page (1963) by partial obstruction of the renal circulation. They demonstrated an increase in the renal venous blood of a pressor polypeptide most likely being angiotensin only 5 min after minor reductions in mean arterial pressure. Obstructions giving equal reductions in pulse pressure and renal blood flow but with less influence on mean arterial pressure did not increase the release of pressor material.

In view of the above reasoning and in accordance with other available data previous and present results may be interpreted by the following hypothetical sequence of events. Any reduction in filtration pressure (e. g. reflex spasm of the afferent arteriole, obstruction of the renal artery, etc.) or any reduction in proximal intratubular pressure induced by a primary increase in the rate of reabsorption will increase the release of angiotensin by some chemical, mechanical or electrical change at the site of the macula densa resulting from the minor decrease in proximal intratubular pressure. Consequently the rate of proximal reabsorption is depressed and thereby lowers the glomelurar filtration rate. The constriction of the efferent arteriole and the depressed rate of reabsorption tend to maintain unchanged the proximal intratubular pressure. With further reduction in the filtration pressure the release of angiotensin increases until a maximum inhibition of the proximal salt T max is reached. Even if much more angiotensin is released with still further drop in the filtration pressure, no further depression of the rate of reabsorption will occur. Thus relatively more of the filtrate is reabsorbed in the proximal tubules and less will reach the distal convolutions. The distal intratubular pressure therefore decreases significantly, as demonstrated in partially clamped kidneys. The reduced internal diameter of the distal tubules in this state of function represents an increased resistance to the luminal flow, which readily explains how the proximal intratubular pressure can be maintained in spite of decreased flow rates in the proximal as well as in the distal tubules. If the filtration pressure drops further, less fluid will filter than can be reabsorbed and the tubules occlude.

An efficient regulation of the proximal intratubular pressure by a factor which acts on the proximal rate of fluid reabsorption (and postglomerular vascular tonus) and thus on the glomerular filtration rate would explain why considerable changes in the latter may occur without significant alterations in the electrolyte excretion rates even though a major fraction (normally about 80 %) of the filtered volume is reabsorbed proximally by a T max type of process. The reason is that the capacity of this process is regulated in a way that tends to maintain constant the proximal hydrostatic pressures. This would imply a fairly constant supply of salt to the distal part of the nephron independent of variations in glomerular filtration rate unrelated to the electrolyte balance, whether these variations were induced primarily by metabolic changes in the tubular transport capacity or by vascular changes in filtration pressure.

According to the proposed hypothesis the physiological function of the juxtaglomerular apparatus is to serve as a feed back mechanism whereby a change being a function of the proximal intratubular pressure (i. e. the out put of the regulated system) at the site of macula densa and via the release of angiotensin changes the proximal salt reabsorption and adjusts the intrarenal vascular resistances in such a way that the proximal intratubular hydrostatic pressure is maintained.

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Acute Changes in Capillary Filtration and Diffusion in Experimental Burn Injury

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Abstract

Arturson G and S Mellander *Acute chan s in cap llary filtration and d ffusion n experimental burn injury* Acta physiol scand 1964 62 457—463 —The effects of second degree burns on the resistance vessels on the net transcapillary fluid movement and on the capillary filtration coefficient (CFC) were studied in the paw of the cat In the acute phase of burn injury there was a pronounced dilatation of the resistance essels a very rapid loss of intravascular fluid to the injured tissue (edema formation) but only a moderate increase of the capillary filtrat on coeffi ient (reflecting capillary permeability) These findings indicate that the edema formation in the ea liest phase of burn njury must be attributed mainly to some other factor than in creased capillary permeability and the consequent leakage of plasma p oteins into the tissue Plasma osmolality in venous blood dra ning the egion and tissue fluid osmolality were found to be increased s gnificantly abo e the control levels during the ea ly phase of burn injury This seems to indicate that during burning a temporary transcap llary osmotic pressure grad ent is established and that the ea ly edema formation is mainly dependent upon a diffus on process wh ch rap dly causes fluid to escape f om the blood stream into the extravascula space of the burned tissue

The most important sign in the acute phase of second degree burns is the marked edema formation in the injured tissue The pathogenesis of this burn edema has there fore been subjected to intense research. Since the edema is produced by an abnormal local accumulation of fluid lost from the circulatory system it is understandable that interest has been focused around problems concerning the anatomical barrier between blood and tissue i e the capillary membrane Such investigation has led to the con clusion that there is a considerable increase of capillary permeab l t in burned tissue because of this an increased leakage of plasma proteins to the injured tissue would occur which in turn would offset the normally existin Starl ng equilibrium across the cap llary wall Such a disturbance has therefore been considered as the main factor respons ble for the edema formation in burns

Most earlier studies of the changes in capillary permeability in burns have been performed by measuring the disappearance from the blood stream and/or the occurrence in lymph or interstitial fluid of *different test molecules* which have been added to the blood stream such as labelled proteins Evans blue dextran etc (Netsky and Leiter 1943 Cope *et al* 1944 1948 1949 Sevitt 1958 Arturson 1961) From these investigations it is however difficult to get any quantitative estimate of the rate of transcapillary fluid transfer in burned tissue For a more complete understanding of the pathogenesis of burn edema it seems necessary to know not only the extent of capillary damage but also what this means in terms of net fluid transfer across the capillary walls This problem may be approached by studying simultaneously the capillary permeability and the rate at which fluid is accumulated in the tissue in the acute phase of a burn

In the present investigation an attempt was made to study quantitatively the acute circulatory changes occurring in second degree burns in cats A technique was utilized which permitted continuous recording of the reactions within the resistance vessels and the changes in transcapillary net fluid movement Further by determinations of the capillary filtration coefficient (CFC) changes of capillary permeability could be deduced

A preliminary report on this study has been published previously (Mellander 1963)

Methods

Observations were made on 20 cats ranging in weight from 2 9 to 4 5 kg anesthetized i v with a mixture of chloralose (50 mg/kg) and urethane (100 mg/kg) The effects of burning were studied in the skin of the hind paw The main principles of the experimental technique (previously used for skeletal muscle) and the method of analysis of the recordings are described in detail elsewhere (Mellander 1960)

The hind paw was enclosed in a water filled temperature regulated plethysmograph to permit continuous recording of tissue volume Blood flow was measured by a drop recorder unit connected to the great saphenous vein at the level of the ankle Other veins and the lymph vessels were ligated at the ankle Arterial inflow pressure and venous outflow pressure of the region were recorded and could be kept constant if desired The capillary filtration coefficient CFC (ml fluid filtered in 1 min and 100 g tissue per mm Hg transcapillary pressure gradient) was determined by increasing the venous outflow pressure (Pappenheimer and Soto-Rivera 1948 Mellander 1960) to levels well above tissue pressure (see Kjellmer 1964) For details concerning the calculation of CFC see Cobbold *et al* 1963 In 5 of the experiments the vasomotor nerves to the paw were left intact in the others they were cut prior to the burn

A second degree burn was produced by exposing the paw enclosed in the plethysmograph to water of 75 C for 20 sec This was done by a temporary exchange of the water in the plethysmograph The temperature of the water in the plethysmograph was kept at 34 C both before and after the burn The burned tissue was examined by histological technique at the end of the experiments

Blood pressure blood flow and volume of the paw were first recorded under normal circumstances and repeated determinations of CFC were performed After this a burn was *produced* maintaining the recording of blood pressure and blood flow The volume recording had to be stopped for about 1 min during this procedure The circulatory responses in the burned hind paw were then followed for 1 to 2 hours including repeated determinations of the CFC

Determinations of the osmolality in plasma and tissue fluid drained from the region were made by thermistor cryoscopy before during and after burning For this purpose repeated samples of venous blood were withdrawn during the experiment Crude samples of tissue fluid were obtained from normal and burned tissues by squeezing the tissue between a pair of cylinders

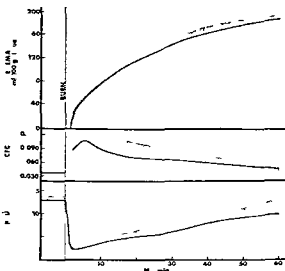


Fig 1 The effects of second degree burns on resistance vessels (PRU) on capillary filtration coefficient (CFC) and on net transcapillary fluid movement (edema) in cat hind paw Solid lines represent means of 5 experiments shaded areas range of individual experiments There is a pronounced dilatation of resistance vessels and a very rapid edema formation especially in the early phase after burning A moderate increase of CFC occurs but this is much less than expected from the rapid transcapillary fluid movement

Results

The diagram of Fig 1 illustrates the vascular effects of a second degree burn in the cat hind paw The effects on the resistance vessels expressed as changes in regional resistance (PRU) on the capillary filtration coefficient (CFC) and on the transcapillary fluid movement expressed as ml of edema fluid accumulating per 100 g of skin tissue are followed for a period of 1 hr after burning The solid curves represent the mean values of 5 expts and the shaded areas indicate the range of individual observations The solid lines happen to describe almost exactly the results obtained in one of the 5 expts and are therefore representative also for the vascular effects observed in one and the same animal In this group of experiments the vasomotor nerves to the paw were left intact In other experiments where the paw was denervated essentially the same results were obtained except that the tone of the resistance vessels was higher prior to the burn

It can be seen that the burn was associated with an immediate and pronounced reduction of regional resistance to flow The dilatation of the resistance vessels was virtually maximal as judged from the fact that a large dose of acetylcholine in this phase was unable to increase blood flow significantly With time there was a gradual return of resistance towards control levels

CFC amounted to about 0 035 in the control period After burning there was a rapid increase of CFC Maximal values (100 to 300 per cent increase) were obtained within 5 to 10 min and then there was a slow gradual decline towards the control level

Despite this moderate increase of CFC burning was associated with a very rapid transcapillary movement of fluid into the extravascular space noted as a progressively increasing edema formation (see Fig 1) The rate of fluid accumulation was highest in the early phase a few min after the burn amounting to 20—40 ml/min/100 g tissue (for technical reasons tissue volume could not be recorded during the first min) With

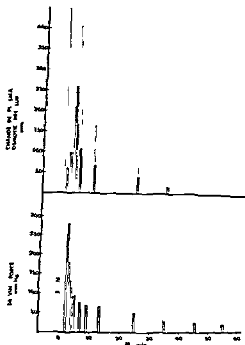


Fig 2 Lower diagram The magnitude of the transcapillary driving force for fluid transfer in burn as calculated from the data on CFC and the rate of edema formation given in Fig 1 (see text) Mean values and range of individual experiments indicated Upper diagram. Change above control level of plasma osmotic pressure in venous blood drained from burned tissue Mean values of 10 experiments and range of individual observations indicated The results suggest that the main factor responsible for burn edema in the early phase is a temporary increase of extravascular osmotic activity

time there was a gradual decline but yet in less than 20 min the tissue had doubled its original weight due to this fluid transfer

When the magnitude of the filtration coefficient and the rate of transcapillary net movement of fluid are known data are available to permit an approximate calculation of the magnitude of the force (expressed in mm Hg) that would be required for this fluid exchange Such deduced values of the driving force based on the data in Fig 1 are shown in the lower histogram of Fig 2 which also indicates the range in the individual experiments During the initial period after the burn the effective driving force required to explain the fluid loss to the burned tissue would amount to 250—300 mm Hg It then rapidly decreases to approach values around 20 mm Hg within an hour after the burn

In 10 cats the osmolality in plasma of the venous blood drained from the burned tissue was determined In all experiments there was a clearcut increase of plasma osmolality above control levels (up to 24 mOsm/kg H_2O) during the first 5 to 10 min after burning and then a fairly rapid return to pre burn control values The results of these experiments are shown in the upper histogram of Fig 2 where mean values and the range of individual observations are indicated The data are here converted from mOsm to osmotic pressure in mm Hg It can be seen that the changes in plasma osmolality observed during the first 10 min after the burn corresponded to *osmotic forces* up to 250—300 mm Hg being in the same range as the driving force indirectly calculated above Admittedly the range of individual observations is great but the general tendency for increase in plasma osmotic pressure is evident The implication of these results will be discussed below

In 2 cats osmolality was determined in tissue fluid squeezed out from burned and normal skin. There was an increase of the osmolality in the fluid from the burned tissue compared to that from normal tissue corresponding to an osmotic force of 374 mm Hg and 476 mm Hg respectively

Discussion

The present investigation has demonstrated that an acute second degree burn is associated with a very rapid transfer of fluid from the intravascular to the extravascular space of the injured tissue in spite of a rather moderate concomitant increase of the capillary filtration coefficient This finding deserves emphasis since it may elucidate to some extent the pathogenesis of burn edema

In burned tissue a disturbance of the normally existing Starling equilibrium across the capillary membrane may occur Firstly owing to a decrease in the ratio of pre to postcapillary resistance associated with the induced regional vasodilatation mean hydrostatic capillary pressure and thereby effective filtration pressure can be expected to increase slightly (*cf* Mellander 1960) Secondly effective plasma protein colloid osmotic pressure would decrease to some extent due to an increased leakage of plasma proteins into the injured tissue (Netsky and Leiter 1943 Arturson 1961) However from these studies it can be estimated that the transcapillary pressure gradient established by these factors must be small and it could in the present experiments hardly exceed 15—20 mm Hg With a pressure gradient of this magnitude the rapid transcapillary movement of fluid observed in the present study could be explained only if there was simultaneously a very pronounced increase of regional capillary permeability Such an increase should have been reflected in the present investigation as a marked augmentation of the filtration coefficient If a transcapillary pressure gradient of 20 mm Hg is assumed CFC should have increased from the control value of 0 035 up to at least 0 5 in the initial phase of burn or by about 1 500 In fact it only increased 100 to 300 Therefore a transcapillary pressure gradient of at least 200 to 300 mm Hg must be assumed to exist in the early phase of a burn as indicated in Fig 2 lower panel Since as mentioned the changes in effective filtration and plasma colloid osmotic pressures can hardly account for more than 1/10 of this value it is therefore suggested that extravascular osmolality in the burned tissue is increased by some mechanism If so fluid would rapidly be lost from the circulation by diffusion until isotonicity was re-established across the capillary membranes Since it is known that small molecules such as H_2O diffuse across the capillary walls at rates very much higher than those at which plasma moves with the blood stream along the capillary channels (Pappenheimer 1953) one might expect an approximate osmotic equilibrium to be established between tissue flu d and plasma after a single passage of blood through the capillary bed. If this occurs the osmolality in plasma drained from the burned region might approach the level of extravascular osmolality

The data presented in Fig 2 seem to strongly support the hypothesis of increased extravascular osmolality in burn Plasma osmotic pressure was found to be raised significantly (upper panel) and roughly to such levels that were predicted by calculating the transcapillary driving force from the data on CFC and the rate of edema formation (lower panel) Further support for this hypothesis is lent by the findings

that the osmolality of "tissue fluid was considerably higher in burned than in normal tissue It should be noted that a sudden increase in the osmolality of the interstitial fluid from the normal level of about 300 mOsm to say 315 mOsm creates a temporary transcapillary pressure gradient of as much as 255 mm Hg It is evident that the changes in fluid transfer across the capillary membrane described above can be explained by alterations of extravascular osmotic pressure that in relation to total osmotic pressure are comparatively small

From the results described it seems justified to conclude that the edema formation in the acute phase of a second degree burn is dependent mainly upon a diffusion process due to a transcapillary osmotic pressure gradient created by the thermic lesion No doubt there is some increase of capillary permeability in burn as suggested also in the present investigation by the augmentation of CFC Part of this increase of CFC may however be ascribed to a dilatation of precapillary sphincters which by increasing the number of capillaries open to flow increases the size of the capillary surface area available for capillary exchange (see Cobbold *et al* 1963) The present *results thus seem to indicate that an increased capillary permeability per se* and the direct effects of this as outlined in the introduction are factors of minor importance for the extremely rapid edema formation seen in the early phase of a burn It is however probable that such factors play a more significant role in the slower edema formation that occurs in later stages of burn injury and to a great extent account for the leakage of plasma proteins into burned tissues

It is beyond the scope of the present investigation to analyse the mechanisms responsible for the increased extravascular osmotic pressure in burns It may however be suggested that instead of selectively damaging the capillary membrane as has often been assumed the burn might to a great extent affect the cellular membranes permitting release of intracellular molecules into the interstitial fluid Such agents may directly or after enzymatic decomposition temporarily raise extravascular osmolality It is known that there is a considerable increase of proteolytic and other enzymes in the tissue after burn trauma (Menkin 1956 Ungar 1960 Rocha e Silva *et al* 1960) Further it is possible that complex compounds from the intracellular space may become dissociated when released into the interstitial fluid Such factors may all contribute to increase the osmotic activity in the interstitial fluid during burn injury

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Eccles and Sherrington (1931) confirmed and extended a lier obser ation on the prolonged period of depression of a flexor reflex when it s a nditioned by a single volley in a contralateral cutaneous nerve At short intervals th ong Lasting depression is complicated by the presence of postsynaptic effects evoked f om ralateral cutaneous afferents Perl (1957) showed that activity in low threshold cutar us afferents produced facilitation in contralateral ankle and knee flexor motoneur nes and at the same time could depress contralateral extensor motoneurones H er an increase in stimulus strength gave prolonged inhibition of flexor motone rones after an initial period of facilitation The early component of these actions w re enerally short (about 25 msec) (also Holmqvist 1961) but the late actions v er present f r 300 msec (Perl 1957) It will be shown below that these prolonged inhibitions are attributable to presynaptic inhibition

Methods

The cats used throughout this invest gation were lightly anesthetized either w th nembutal urethan -chloralose S nce no difference was detected n the results from eithe r pe t naest s a all the results were pooled together The pure sk n nerves sural (SUR superficial pe n al (SP) occas onally the saphenous (Saph) and the nerve w th an admixture of muscl and skin afferents posterior tibial (PT) were dissected in both hindl mbs A lam nect m as made f om lumbar one to lumbar seven level and the cord was transected at lumbar one

The five methods of invest gation have already been described in detail in publ ations from this laboratory on ips lateral presynapt c inhibit on (Eccles Magni and W llis 196 Eccles Kostyuk and Schmidt 1962 a b Eccles *et al* 1963a) recording from the central end of a dorsal root filament the depolarizations electronically sp eading from the intramedullary course of this filament i e the DRP record ng presynapt c depolarizat on intracellularly with high resistance 3M KCl microelectrodes from primary afferent fib es primary afferent depolarization PAD) recording changes n excitability of primary afferent fibres in the spinal cord invest gating the effect of conditioning volleys on the flexor reflexes rec rded n the cut central ends of the ventral roots and finally recording intracellularly from motoneurones n order t obta n the effects reported on flexor reflexes in s ngle cells Here the same methods a e employed to test the effectiveness of contralateral cutaneous volleys and in another paper contralateral muscle volleys (Eccles Holmqvist and Voorhoeve 1964 and contrast them with volleys in the ipsilateral nerves

The abbreviat ons emploved for the skin nerves are given abo e The psilateral ural is deno ed as i Sur and 4 volleys as 4 i Sur Contralateral nerves are ind ated by a small c i e contralateral Sural as c Sur Due to the depression of p esynaptic inh bition at high f equencies all rec rds were obta ned by the superpos t on of several records at 1—2 per sec The t me con tant of the ampl fier was usually 0.3 sec, but when record ng dorsal root potent als a time constant of 1 0 sec wa employed

Results

Io ipsila eral and cont alateral cu.aneous nerves

of the central terminals of afferent fibres may along the dorsal roots A small dorsal root s or lower lumbar s x root is isolated from the then lifted onto electrodes one being on the mm to the cord entry Barron and Matthews a by purely cutaneous or muscle afferent Fuortes 1952 Eccles and Krnjevic 1959 les Magni and Willis 1962 Eccles *et al*

Acta physiol scand 1964 62 464—473

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Presynaptic Inhibition from Contralateral Cutaneous Afferent Fibres

By

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Abstract

Eccles R M B Holmqvist and P E Voorhoeve *Presynaptic inhibition from contralateral cutaneous afferent fibres* Acta physiol scand 1964 *62* 464—473 — Depolarization of the cutaneous nerve terminals by volleys in any contralateral cutaneous nerve has been shown by three experimental procedures by electrotonic spread of the depolarization along the dorsal root fibres by intracellular recording from primary cutaneous afferents and by excitability changes at the fibre terminations These crossed presynaptic inhibitions were usually less powerful than those from the ipsilateral cutaneous nerves and had a longer latency 6 msec instead of 3 and a maximal effect at 30—50 msec instead of at 15—20 msec Like in the ipsilateral cutaneous nerves maximum effects with conditioning volleys in cutaneous fibres were obtained with volleys of about four times threshold

Many types of presynaptic inhibition have been investigated in the cat spinal cord muscle afferents onto muscle afferents (Frank and Fuortes 1957 Frank 1959 Eccles Eccles and Magni 1962 Eccles Schmidt and Willis 1963a b and c) cutaneous onto cutaneous (Eccles Kostyuk and Schmidt 1962a b and Eccles Schmidt and Willis 1963d) muscle afferents onto cutaneous and cutaneous onto muscle afferents (Eccles et al 1963d) All of these investigations employing conditioning by afferent volleys into the spinal cord have been restricted to ipsilateral actions This present investigation has been concerned with the effects of conditioning volleys on the contralateral side

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Eccles and Sherrington (1931) confirmed and extended earlier observations on the prolonged period of depression of a flexor reflex when it was conditioned by a single volley in a contralateral cutaneous nerve. At short intervals this long lasting depression is complicated by the presence of postsynaptic effects evoked by contralateral cutaneous afferents. Perl (1957) showed that activity in low threshold cutaneous afferents produced facilitation in contralateral ankle and knee flexor motoneurones and at the same time could depress contralateral extensor motoneurones. However, an increase in stimulus strength gave prolonged inhibition of flexor motoneurones after an initial period of facilitation. The early component of these actions were generally short (about 25 msec) (also Holmqvist 1961) but the late actions were present for 300 msec (Perl 1957). It will be shown below that these prolonged inhibitions are attributable to presynaptic inhibition.

Methods

The cats used throughout this investigation were lightly anesthetized either with nembutal or urethane-chloralose. Since no difference was detected in the results from either type of anaesthesia, all the results were pooled together. The pure skin nerves sural (SUR), superficial peroneal (SP), occasionally the saphenous (Saph), and the nerve with an admixture of muscle and skin afferents, posterior tibial (PT), were dissected in both hindlimbs. A laminectomy was made from lumbar one to lumbar seven level and the cord was transected at lumbar one.

The five methods of investigation have already been described in detail in publications from this laboratory on ipsilateral presynaptic inhibition (Eccles, Magni and Willis 1962; Eccles, Kostyuk and Schmidt 1962 a, b; Eccles *et al.* 1963a): recording from the central end of a dorsal root filament the depolarizations electrotonically spreading from the intramedullary course of the filament, i.e. the DRP; recording presynaptic depolarization intracellularly with high resistance 3M KCl microelectrodes from primary afferent fibres (primary afferent depolarization, PAD); recording changes in excitability of primary afferent fibres in the spinal cord; investigating the effect of conditioning volleys on the flexor reflexes recorded in the cut central ends of the ventral roots; and finally recording intracellularly from motoneurones in order to obtain the effects reported on flexor reflexes in single cells. Here the same methods are employed to test the effectiveness of contralateral cutaneous volleys and in another paper contralateral muscle volleys (Eccles, Holmqvist and Voorhoeve 1964) and contrast them with volleys in the ipsilateral nerves.

The abbreviations employed for the skin nerves are given above. The ipsilateral sural is denoted as Su and 4 volleys as 4 Sur. Contralateral nerves are indicated by a small c, i.e. contralateral Sural as c Su. Due to the depression of presynaptic inhibition at high frequencies all records were obtained by the superposition of several records at 1—2 per sec. The time constant of the amplifier was usually 0.3 sec, but when recording dorsal root potentials a time constant of 1.0 sec was employed.

Results

Dorsal root potentials evoked by volleys in ipsilateral and contralateral cutaneous nerves

A measurement of the depolarizations of the central terminals of afferent fibres may be obtained from the electrotonic spread along the dorsal roots. A small dorsal root filament in either upper lumbar seven or lower lumbar six root is isolated from the rest of the root, cut peripherally and then lifted onto electrodes, one being on the peripheral cut end, the other as close as 1 mm to the cord entry (Barron and Matthews 1938). The dorsal root potentials generated by purely cutaneous or muscle afferent volleys have been examined by Brooks and Fuortes 1952; Eccles and Krnjević 1959; Eccles, Kostyuk and Schmidt 1962a, b; Eccles, Magni and Willis 1962; Eccles *et al.* 1963a.

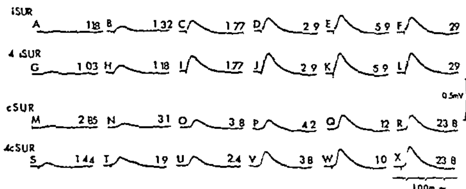


Fig. 1. Dorsal root potentials recorded in the central end of a filament of L7 dorsal root to single (A–F, M–R) and 4 volleys (G–L, S–X). The effect of contralateral SUR (M–X) is compared to ipsilateral SUR (A–L). Numbers on each record indicate strength of stimulus. The same voltage scale (0.5 mV) and time scale (100 msec) applies to all records.

In Fig. 1 in the same L7 dorsal root filament the dorsal root potentials (DRPs) were evoked by single or repetitive volleys applied to the ipsilateral SUR nerve (first two rows) or the contralateral SUR nerve (lower two rows). It is observed that ipsilateral SUR is an effective depolarizer at quite low stimulus strengths (Fig. 1B, 1.32T) whereas stronger stimuli are required for the contralateral SUR (Fig. 1N, 3.1 T). Ipsilateral SUR shows the same characteristics as in other investigations, i.e. in the first row it is seen virtually to reach a maximum at 2.9 T, there being little addition with a volley of 29 times threshold (F). In the third row contralateral SUR does not reach a maximum at 4.2 times threshold (P). A maximal DRP was obtained with a stimulus strength of 5T (not illustrated). A stimulus of 12T (Q) evokes a DRP which is the same as that when the stimulus strength is doubled (i.e. 23.8T in R). Consequently 4 times threshold may be considered maximal for single volleys in ipsilateral cutaneous nerves though a stronger stimulus may have to be applied to contralateral skin nerves to evoke maximal effects.

The DRPs from iSur are slightly larger than those evoked by cSur but also have a shorter latency, a steeper rate of rise to an earlier summit and appear with weaker stimuli (Fig. 1A at 1.18T and Fig. 1M at 2.85T). Dorsal root reflexes (DRR s) are superimposed on the DRPs from iSur at high strengths of stimulation (Fig. 1E, F, K and L). *It is interesting to note that four volleys (at 400/sec) to a cutaneous nerve are* more effective than a single stimulus (cf. Fig. 1D with J or P with V). The increase for the ipsilateral nerve is unusually large, as a rule the increase is less than 20%. On the other hand there are always large increases with repetitive stimulation of contralateral cutaneous nerves. Since DRPs only indicate that some contralateral primary afferents are depolarized by cutaneous afferents it is necessary to define the *modality* of the afferent fibres depolarized. This requires the more precise measurements of intracellular recording and excitability testing which will be described in the next sections.

Intracellular recording from cutaneous afferent fibres

As has already been described (Eccles *et al.* 1963d) volleys in ipsilateral cutaneous nerve fibres evoke depolarizations in cutaneous fibres belonging to the same or another

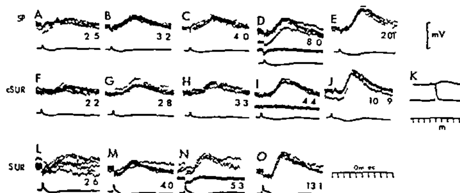
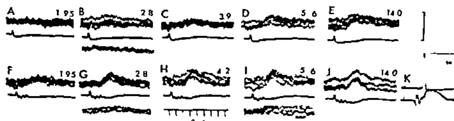


Fig 2 The upper records (A J L-O) were obta ned from a microelectrode inside an SP afferent fibre (identified in K) The lower traces are recorded by an electrode making contact with the L6 dorsal root as it enters the spinal cord negati ity being signalled upwards A E are records of the primary afferent depolarization induced by single volleys in contralateral SP on increasing the st ength from 2 5 to 20 times threshold F J are the depolarizat ons produced by cSur From L-O are the records for ipsilateral Sur The 10 msec time and mV potential scale apply only to the primary afferent depolarizat ons In certain records (D I N) a third reco d (in between intracellular and entering volley records) is shown to ind cate the size of the fields just extracellular to the fibre



F g 3 Intracellular records (upper traces) fro n a PT fibre (ident fied n K) to show the effect of ingle (A E) compa ed to 4 olleys (F J) appl ed to the contralateral Sural nerve Strengths of st mul t on are ndicated on each reco d Re ords obtained just outside the fibre are illustr ted in the lowest ecord of B G and I Potential scale (mV) appl es only to the primary aff rent depol ri ations 10 mse t m scale for A J and msec time scale for K.

cutaneous nerve In this present series the effects of contralateral and ipsilateral cutane ous volleys v ere compared In Fig 2 single volleys in the contralateral SP (A E) and *SUR nerves (F J) evoked prolonged depolarizations of a fibre identified as an SP fibre* in K Gradual increase in stimulus strengths caused these primary afferent depolariza tions (PADs) to become larger until a maximum was obtained usually between 4—6 times threshold (Fig 2D and I) for contralateral nerves and 4 times threshold for ipsilateral (Fig 2M and N) The ipsilateral SP nerve contained the fibre under ob- servation so after potentials following its action potential were superimposed on the synaptically induced d polarization, which was therefore not illustrated The middle record of the extracellular potential (Fig 2D I and N) just outside the fibre indicates

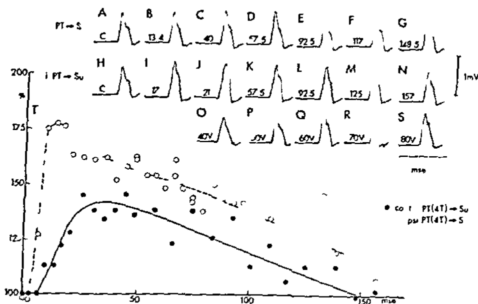


Fig 4 A low resistance (1 MΩ) microelectrode filled with 4M NaCl solution was inserted into the cord to a depth of 1 7 mm from the cord dorsum Recording from the microelectrode (not illustrated) showed the tip to be in the neighbourhood of the area where the lowest threshold cutaneous afferent fibres terminate on interneurones Single pulses (0 2 msec in duration) from the Grass stimulator with an isolation unit activated nearby dorsal afferent fibres These impulses were transmitted antidromically along the fibres to the hind limb where they were recorded in the SUR nerve Conditioning with single volleys (4 times threshold) in either the contralateral (B-G) or the ipsilateral (I N) PT nerve increased the excitability so that larger action potentials were recorded (cf C and A or I and H) The numbers on each record give the interval between conditioning and test volley in msec The calibration series (O S) shows the size of the impulse evoked by the voltages indicated The full time courses of these effects are shown in detail (Fig 4T) where the x-abscissae gives the time in msec between conditioning and test volley and the ordinate the percentage increase of the recorded potential compared with the control response to 50V Details of this technique have already been given (Eccles Magni and Willis 1962

that the potentials observed in the upper records of Fig 2D I and N have only a very small field potential hence they are virtually all due to a membrane potential change of the fibre i e to a PAD

In Fig 3 the effects of single and repetitive activation were investigated since summation increased contralateral effects e g DRPs (see above) The difference between single and repetitive stimulation of an ipsilateral cutaneous nerve is less (Eccles *et al* 1963d)

Testing excitability of nerve fibres

A more indirect method of investigating depolarization of the afferent fibres is provided by tests of their excitability This method developed by Wall (1958) has the advantage of demonstrating any alterations in the excitability of specific types of afferent fibres without requiring the difficult and tedious intracellular techniques This method

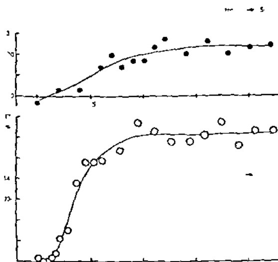


Fig 5 With the same recording as Fig 4 fixed intervals were chosen for the conditioning volleys (17 msec for ipsilateral PT and 44 msec for contralateral PT) The strength of stimulus applied to the conditioning nerve was altered over the range 1 0 to 3 5 times threshold and the results are plotted in Fig 5 the upper curve for cPT and the lower for iPT

gives a statistical evaluation of excitability changes of afferent fibres and has been extensively employed for various types of large afferent fibres on the ipsilateral side (Wall 1958 Eccles Magni and Willis 1962 Eccles *et al* 1963a d) In Fig 4 the increases in excitability produced by a conditioning volley in contralateral PT (A G) were less than those evoked by ipsilateral PT (H N) where the maximum at 17 msec (i e I) was 75% The percentage was estimated by comparing the spike size with those evoked by different known voltages This is called the calibration curve and is shown in Fig 4 from O S Therefore conditioning by the contralateral cutaneous volleys caused an increase in excitability of the primary cutaneous afferent fibres in the spinal cord which in general resembled that produced ipsilaterally but was smaller and slower For example in Fig 4T the full time courses are shown for conditioning volleys in cPT and iPT of which a few responses were illustrated in Fig 4A C and H N The contralateral PT nerve was less effective than the ipsilateral the latency was about 5 msec longer the maximal effect reached later at about 40 msec compared to about 15 msec from the ipsilateral side and the total duration of both was about 150 msec

In order to test the relationship between strength of stimulation of the conditioning nerve and the increased excitability of primary afferent fibres optimal test intervals were selected (17 0 msec for the ipsilateral and 44 msec for the contralateral In Fig 5 it can be seen that 90 of the effect was achieved in both contralateral and ipsilateral nerves by single volleys at a strength of 3 threshold Also in Fig 5 conditioning by an ipsilateral volley caused a change in excitability larger than that produced contralaterally 60 as against 25

Fig 6 illustrates another investigation on the effectiveness of conditioning volleys in ipsilateral and contralateral nerves at the optimal test intervals. The results employing either a single or 4 volleys at 200 sec are summarized graphically in J where it may be observed that the contralateral nerves (cSur cSP cPT produced smaller increases

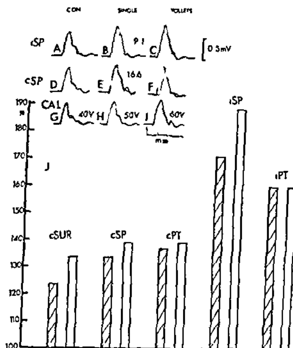
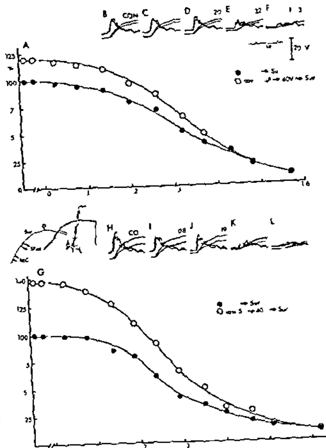


Fig 6 The effect on excitability of sural afferent fibres was altered by one or four conditioning volleys in the ipsilateral and contralateral cutaneous fibres The most effective intervals were chosen i e 17 msec for ipsilateral nerves and 44 msec for contralateral The maxima for the three contralateral nerves (cSur cSP cPT) whether employing single (hatched) or 4 volleys (unhatched) are much less (Fig 6J) than for the iSP or iPT nerves even though stimulus strengths were greater 9 1 for iSP and iPT and 19 2 16 6 20 8 for cSur cSP and cPT respectively Specimen records above J show in A C the effect of iSP compared to cSP D F G I are calibrat on records for series All records at the same potential and time scales

in excitability than the corresponding ipsilateral nerves (iSP and iPT) Examples of the responses used in constructing J are given above in A F where a single volley (B) and four volleys (C) in ipsilateral SP were compared to the increased spike sizes evoked by the increase in voltages from 40V to 50V (H) and 60V (I) i e the calibration series The increase is also given for the control 40V (D) by 1 or 4 impulses at 16 6 times threshold in cSP (E and F respectively)

In order to investigate the type of fibre which is stimulated by the extracellular microelectrode in these excitability tests a second pair of electrodes was placed under the sural nerve (inset in Fig 7) so as to apply the collision technique that has been used in separating the Ia and Ib responses (Eccles *et al* 1963a) In Fig 7B or H the response to a pulse of 40V applied to the microelectrode in the dorsal horn evoked a spike discharge which was recorded in the sural nerve in the periphery either as the upper traces in each of the two components of Fig 7B and H or the integrated records (the lower traces) Conditioning with either contralateral (C F) or ipsilateral (J L) SP volleys increased the spike discharge (the upper record or upper integrated records in Fig 7B or H) If however a stimulus was applied to the second pair of electrodes on the sural nerve the interval could be so arranged that impulses in the fibres travelling orthodromically into the spinal cord collided with the antidromic impulses initiated by the pulse through the microelectrode in the dorsal horn (see inset Fig 7) Fig 7B and H may be taken as representative of the facilitated and unfacilitated discharges Increase in the strength of stimulus evoking the colliding volley from 1 08T to 1 40T diminished the spike discharges (Fig 7F and L) The full courses for the facilitated

Fig 7 A second pair of electrodes have been placed under the sural nerve so that one pair may be used to record the impulses transmitted from the spinal cord following activation of the dorsal fibres and the other pair may be used to stimulate the sural nerve just before the antidromically conducted impulses from the cord reach the recording electrodes (see inset) The voltage from the Grass stimulator was kept constant at 40V the intervals between the conditioning volleys and the pulse were constant about 17 msec for cSP and 4 msec for iSP the only variable was the strength of stimulus applied to the sural nerve to produce collision which varied from 1 08 to 1 6 times threshold The 20 μV potential scale applies to the upper traces B-F H L the time scale to all records The series B-F is plotted in A and the series H L in G



and unfacilitated discharges are plotted in Fig 7A for contralateral SP and in Fig 7G for ipsilateral SP as conditioning stimulus Therefore the collision technique shows that both the unfacilitated and facilitated impulses travel in the lowest threshold fibres which accords with the results obtained more directl (Fig 6 where the facilitation has been investigated in the fast conducting fibres

Contralateral effects on motoneurones

Motoneuronal activity was measured either by intracellular recording Fig 8 or by the flexor reflexes recorded in the ventral roots not illustrated In a PBST motoneurone the 4 volleys in contralateral PT (3 5T) in Fig 8B reduced the polysynaptic EPSP (A evoked by ipsilateral sural alone at an interval of 37 msec Similarly volleys in cSP (7.2T) i e Fig 8D reduced the control EPSP C though cSP alone Fig 8G did not evoke any postsynaptic potential on the motoneurone itself at the interval chosen 4cPT evoked no postsynaptic potential on the motoneurone at all (Fig 8E There are t o possibilities the contralateral cutaneous volleys could make the ipsilateral

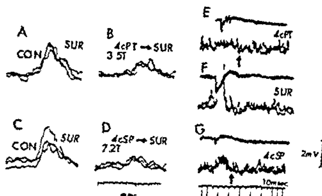


Fig. 8. In a PBST motoneurone the EPSPs induced by an ipsilateral sural volley (A, C) are conditioned by 4 cPT (B) at 3.5 times threshold or by 4 cSP (D) at 7.2 times threshold. The interval between volleys is shown by the arrows in E and G indicating the interval after the conditioning volley at which the testing volley was tried (note 10 msec time scale under G). The msec scale under D applies to records A, B, C and D. E, F, G show the dorsal surface potential on the upper beam, and on the lower, the intracellular potentials to 4 cPT (E), iSur (F), 4 cSP (G). The 2 mV potential scale applies to all intracellular records.

sural less effective either by altering the incoming volley (presynaptic inhibition) or by postsynaptic inhibition of some interneuronal pathway. It must remembered that some interneurones are common to both the cutaneous and the G II and III pathways (i.e. FRA) to the motoneurones at least on the ipsilateral side (Eccles and Lundberg, 1960) and could be common to afferent discharges evoked by stimulation of contralateral G II and III or cutaneous fibres.

Discussion

The observations on presynaptic inhibitory effects from contralateral cutaneous afferent fibres show that the inhibition from contralateral onto ipsilateral cutaneous is not unlike that exerted by ipsilateral fibres onto themselves (Eccles, Schmidt and Willis 1963d). The inhibitions as measured by primary afferent depolarizations (PADs), dorsal root potentials (DRPs) or excitability measurements are not as powerful as those recorded on conditioning with ipsilateral skin nerves. However their time courses are similar though the time for maximal effect is usually 25 msec or so longer.

In a preliminary investigation the interneuronal pathways involved in this presynaptic inhibitory pathway from crossed skin afferents have been studied. No matter how close the microelectrode was to the midline no interneurone was found (out of thirty-five cells) in the base of the dorsal horn that could be stimulated by low threshold contralateral cutaneous volleys though interneurones have been observed that are stimulated by high threshold cutaneous fibres. However since presynaptic inhibitory effects may be obtained with volleys of 1.3 times threshold for contralateral nerves such interneurones cannot belong to a pathway activated by low threshold cutaneous impulses whereas they could conceivably be involved in the pathways connecting high threshold presynaptic effects across the cord. Interneuronal discharges to an ipsilateral cutaneous volley were usually reduced by a volley in any of the contralateral

cutaneous nerves This could be due to presynaptic inhibition on the afferent fibres or to post-synaptic inhibition on the interneuronal membrane Further work is required before the actual internuncial chain can be understood

The physiological significance and the supraspinal control of presynaptic inhibitory pathways are fully discussed in the next paper (Eccles *et al* 1964)

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From the Department of Physiology Australian National University Canberra Australia

Presynaptic Depolarization of Cutaneous Afferents by Volleys in Contralateral Muscle Afferents

By

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Abstract

Eccles, R. M B Holmqvist and P E. Voorhoeve *Presynaptic depolarization of cutaneous afferents by volleys in contralateral muscle afferents* Acta physiol scand. 1964 62 474—484 — Depolarization of cutaneous nerve terminals by all contralateral muscle volleys has been demonstrated by several procedures by intracellular recording from the cutaneous fibres by electrotonic spread to dorsal root filaments so giving the dorsal root potentials (DRP and by threshold changes in the fibre terminals. Groups II and III muscle afferents depolarized cutaneous fibre terminals on both sides of the spinal cord while the Ib volleys were effective only ipsilaterally The crossed actions of group II and III volleys were weaker than the ipsilateral actions and had a longer latency (10—20 msec and a later summit (50—80 msec This depolarization of the central terminals of the cutaneous fibres by afferent volleys from contralateral muscles resulted in a depression of synaptic activity of these cutaneous fibres as evidenced by the flexor reflex the cutaneous dorsal root potential and the afferent cutaneous tract discharge in the spinal cord

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Recent investigations on the spinal cord have demonstrated that afferent volleys have a depressant action upon the efficacy of the synaptic transmission of primary afferent fibres on the ipsilateral side and that this depression is due to a depolarization of the primary afferent fibres with a consequent diminution in the presynaptic spike potential hence the term presynaptic inhibition (Eccles Eccles and Magni 1961 Eccles, Kostyuk and Schmidt 1962c, Eccles Magni and Willis 1962) The present investigation concerns the presynaptic inhibitory action that contralateral muscle impulses exert upon cutaneous nerves An attempt was made to determine which afferent fibres were responsible for these effects that is whether Group Ia Ib II or III fibre groups of extensor or flexor muscles are involved.

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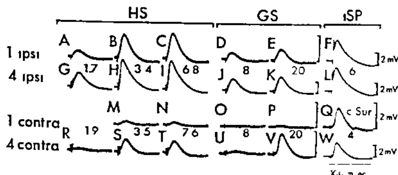


Fig 1 Dorsal root potentials (DRP s) recorded in an SI dorsal root fila it he e th r a s n le volley (A E M P) or a train of 4 volleys (250 sec) (G-K R V) was applied t th psi late al and contralateral HS GS nerves as indicated by column head The p tent al scales of 2 mV for each row are displayed at the end of records E K P and V F d L ar the DRP s evoked by 1 or 4 volleys in iSP and Q W are the DRP s to 1 or 4 lle s n Su Each record (F L Q W) has its o n potential scale Stimulus strengths exp essed multiples of threshold for the nerves are given between each pa r of records The 100 msec t me s al applies to all records

Methods

Th experiments were performed on cats l ghtly anesthetized with nembutal The spinal co d was transected at the upper lumbar level The ventral roots L5—S? v er cut on the s de used f r testing The experimental procedure resembles those prev ously eported in comparable investigations from this laboratory (Eccles Kostyuk and Schm dt 196?b Eccles Magni and Willis 196?) For repetitive stimulation it was found that 4 volleys at 200—300 sec usually caused maximal effects on the contralateral side as well as on the ps lateral de

The crossed actions were tested on two cutaneous nerves sural (Su) and superficial peroneal (SP) and on the posterior t bial (PT) which is predom nantly cutaneous but w th an admi ture of muscle afferents For cond t oning the follo ng nerves we e bilaterally dissected and w ll be referred to by the following abb eviations posterior b ceps and emitendinosus (PBST but sometimes with the addit on of semimembranosus and anterio b ceps as th hamstring n rve (HS) gastrocnemius-soleus (GS) plantaris (Pl) flexo d gitorum longus and flexo hallucis longus (FDHL) extensor digitorum longus plus tib alis ant rior (DP) quadriceps Q and the nerve to the knee jo nt (J)

Results

Dorsal root potential

The simplest method of demonstrating the depolarization of primary afferent fibres is to record it in dorsal root filaments after electrotonic spread from the depolarized afferent nerve terminals (Barron and Matthews 1935 1938 Bremer and Bonnet 1942 Lloyd and McIntyre 1949 Bernhard 1952 1953 Koketsu 1956 Eccles Kostyuk and Schmidt 1962a Eccles Magni and Willis 1962) Fig 1 demonstrates dorsal root potentials (DRPs) recorded when a single and a train of 4 volleys at 200 sec were applied to contralateral HS and GS nerves It was found that these contralateral DRPs (lower two sets of records M P R V) have similar time courses though they were much smaller than those produced by corresponding volleys in ipsilateral HS and GS nerves (upper two sets of records A E G K) (cf Eccles Schm dt and Willis 1963b) For comparison the DRPs evoked by volleys in cutaneous ipsilateral SP nerves

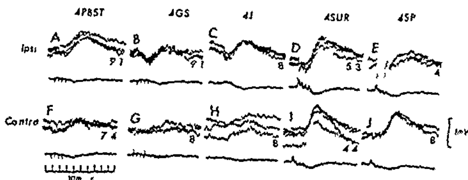


Fig 2 Intracellular recording of primary afferent depolarization in an SP fibre (id ntified in E) A J consist of 2 records the upper is the intracellular record the lower shows the incoming volley recorded from the surface of the cord at the dorsal root entry zone All records are evoked by the application of four volleys and have the same potential and time scales A E are the responses evoked by the ipsilateral nerves as indicated by column headings and F J for the contralateral Stimulus strengths expressed as multiples of threshold for the nerve are given underneath each intracellular record

(F L) and contralateral Sur (Q W) are given Since the contralateral actions from muscle nerves were considerably smaller temporal facilitation proved to be more important and four muscle volleys were found to be more effective than a single The sizes of the DRPs when one stimulus was applied to the ipsilateral HS and GS nerves were usually larger than when a train of impulses of similar strength was applied to the contralateral nerves (cf records A E with R V) but E is smaller than V Due to an inability to stimulate either cSP or iSur the actions of iSP are compared with cSur The DRPs from iSP (F L) were large than those of cSur (Q W) with a shorter latency and an early maximum similar to those illustrated in Fig 1 of Eccles Holmqvist and Voorhoeve (1964) Volleys in Q PI FDHL and DP nerves were found to evoke similar contralateral DRPs Such DRPs demonstrate the existence of depolarization of contralateral fibres but give no indication of the types of afferent fibres that are depolarized The identification of afferent fibre type is possible by testing the excitability changes and by intracellular recording from afferent nerve fibres Wall 1958 Wall *et al* 1956 Eccles Magni and Willis 1962)

Intracellular recording from cutaneous afferent fibres

Intracellular recordings can be obtained from primary afferent fibres if th fibres are *impal d near their entrance to the spinal cord dorsum* (*Eccles and Krnjević 195*) The fibres are *identified by the spike potential that is recorded by stimulating* the appropriate afferent nerve Fig 2 *illustrates intrafibre recording from a* SP fibre which is orthodromically activated by 4 *volleys at 4 times threshold* (E)

As has already been described stimulation of ipsilateral high threshold muscle and skin nerves produces a depolarization (a primary afferent depolarization — PAD) in the cutaneous nerve terminals (Eccles *et al* 1963b) In Fig 2 records A J illustrate the depolarization produced by a train of 4 volleys at 250/sec It was found that volleys applied to the contralateral PBST and GS nerves evoked similar depolarizations (Fig 2F and G) to those evoked by ipsilateral PBST A and GS (B) The depolarization

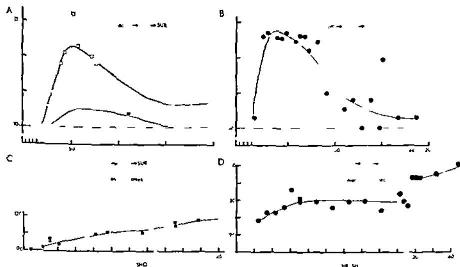


Fig 3 Testing the intraspinal changes in excitability of the sural afferent nerve terminals A microelectrode was inserted into the dorsal horn where the Sural afferents terminate and a test pulse of 40V (0 2 msec duration) was applied through the microelectrode and an indifferent earth the evoked action potential being recorded antidromically in the Sural nerve in the periphery By the standard procedures excitability changes in the sural nerve were determined under the various experimental conditions and are plotted as percentages of the control In the upper graphs A and B the increase in excitability of the Sural fibres by 4 conditioning volleys in HS nerve (ordinate) is plotted with the interval between conditioning and test stimuli as abscissa At an interval giving optimal action (32 msec for ipsilateral and 50 msec for contralateral nerves) the curves of C and D were plotted to relate the increase in excitability of the Sural nerve (ordinate) with the strengths of stimulation relative to threshold of the HS nerve (abscissae)

evoked from contralateral nerve stimulation was usually smaller than the ipsilateral e g that produced by contralateral GS is only a fraction of that evoked by ipsilateral GS Stimulation of the nerves from the knee joint (G and H) shows larger PADs from ipsilateral (G) than contralateral (H) On the other hand the PADs from the two surals (D and I) are of similar magnitude and both are larger than those from muscle and joint

The close parallelism of the PAD produced by ipsi and contralateral volleys strongly suggests that they have a similar mechanism of action The configuration and time course of the crossed depolarization in Fig 2 also resembles the DRP in Fig 1 and it has therefore been concluded that the DRP evoked by volleys in contralateral muscle nerves is partly due to electrotonic spread in depolarized skin afferents Crossed PADs as described above have been recorded intracellularly also from fibres in the Sur and PT nerves

The advantage with the intrafibre recording is that it reveals directly the presynaptic depolarization of the nerve fibre which can be identified The random sampling of actions are of course a limitation of the technique but the greatest disadvantage is the difficulty to keep recording conditions sufficiently constant for further analysis Hence it is not suitable for detailed study and quantitative comparison

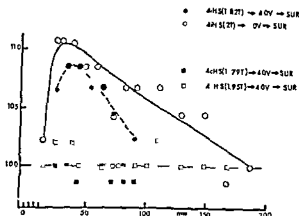


Fig 4 Increase in excitability of the Sural fibres by a train of 4 volleys in the ipsilateral (O ●) and contralateral (□ ■) HS nerves Procedure as described in Fig 3 The stimulus strengths were chosen to be slightly submaximal and slightly supramaximal for group I HS fibres as determined by the incoming volley in the dorsal root entry zone when tested with the conventional double volley technique

Excitability changes in the central nerve terminals

The method most suitable for investigating the pattern of organization of the presynaptic actions is the measurement of excitability changes in the primary afferent fibres For this purpose the spinal afferent nerve terminals were stimulated by current pulses through the microelectrode (Eccles Magni and Willis 1962) For any afferent nerve the number of primary afferent fibres excited is given approximately by the size of the spike potential recorded antidromically in that peripheral nerve and when the threshold is lowered as a consequence of depolarization of these afferent fibres there is an increased size of this antidromic spike potential (Wall 1958 Eccles Magni and Willis 1962)

In Fig 3 a square pulse of 40V was applied through a microelectrode at the depth where conditioning volleys in a contralateral nerve gave the largest increase in excitability The antidromic spike was recorded in Sur nerve As described by Eccles Magni and Willis (1962) it is possible by means of a control calibration curve to determine the average increase in excitability that was responsible for any given increase in the antidromic spike potential In Fig 3A and B the changes of excitability in the Sur fibres were tested at various intervals after conditioning stimuli had been applied to the contralateral (A) and the ipsilateral (B) HS nerves The time course of the excitability changes closely parallelled the depolarization obtained by intracellular recording (Fig 2)

The increases in excitability produced by contralateral volleys were less than with the corresponding ipsilateral volleys and the summits were later An increase in the excitability of the Sur fibres in Fig 3A was detected about 20 msec after the first conditioning volley Crossed actions from muscle volleys have never had latent periods briefer than 10 msec These high values for latency indicate a transmission over several interneurones When volleys in contralateral nerves evoked larger depolarizations the maximum tended to shift forward and appeared slightly earlier though it was always later than the maximal actions evoked from the ipsilateral side

In the graphs in Fig 3A the time course for the contralateral actions from HS was plotted at two conditioning stimulus strengths The weaker stimulus strength at 5 times threshold for the nerve was chosen so that group I and most of group II fibres were

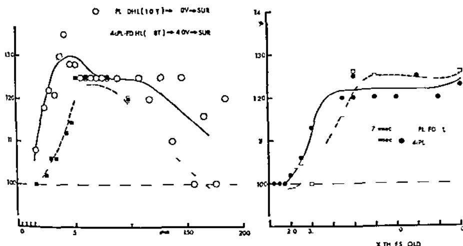


Fig 5 Increase in excitability of the sural afferent fibres by conditioning volleys in ipsilateral (0 ●) and contralateral (■ □) PL-FDHL nerves Procedure as in Fig 3 In graphs to the left the time course is plotted for the increase of excitability at fixed conditioning stimulus strengths as indicated in the figure In graphs to the right the testing interval is fixed at the optimal values given (47 and 30 msec respectively) and the effects of increasing stimulus strengths in the PL-FDHL nerve (abscissae) are plotted against the changes in excitability (ordinate)

activated (Eccles and Lundberg 1959) At this strength the crossed effect was small and more powerful crossed presynaptic actions were usually not obtained until group III fibres were activated In Fig 3C and D the increases in excitability of the Sur fibres are plotted as a function of conditioning stimulus strength The action was tested at the interval giving optimal action (50 msec and 32 msec respectively) As can be seen in Fig 3C there was no noticeable action at a stimulus strength of 2 times threshold for the contralateral HS nerve This strength was slightly supramaximal for group I fibres in this experiment The maximal group I strength was estimated by the size of the incoming volleys in the dorsal root entry zone when the conventional double stimulus technique was applied to the first and sometimes also the fourth volley in the train of conditioning volleys (Bradley and Eccles 1953 Eccles Eccles and Lundberg 1957 Laporte and Bessou 1957) Not until the stimulus strength was raised so that group II fibres were excited at about 2 5—3 times threshold did the excitability increase There was a further addition when group III fibres were included e g at a stimulus strength above 5 times threshold (cf Fig 3C D) (Eccles and Lundberg 1959)

The method of correlating the evoked actions of different fibre groups by means of different stimulus strengths at a fixed test interval has the limitation that maximal actions produced by various fibre groups might appear at different intervals In order to check the group I effects from muscle the full time course was plotted at two stimulus strengths one slightly submaximal (1 79 times threshold) and one slightly supramaximal (1 95 times threshold) for group I (Fig 4) Even under these circumstances no crossed actions were disclosed As a control the ipsilateral group Ib actions onto cutaneous

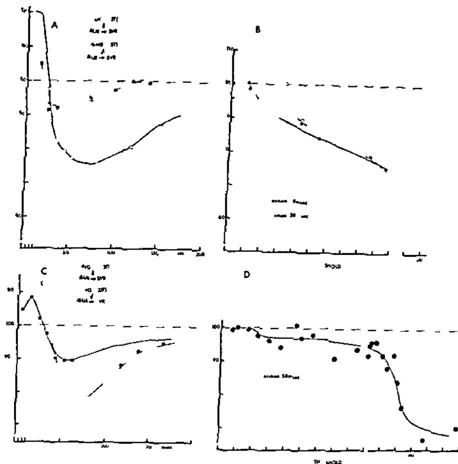


Fig 6 Inhibition of the flexor reflex by a train of 4 voll ys in contralateral HS (A B) and GS (C D) nerves The flexor reflex was evoked by stimulating Sural nerve and the integrated flexor reflex was recorded in the ventral SI root In the graphs to the left (A C) the full time course is plotted for conditioning at two different stimulus strengths as indicated In the graphs to the right (B D) the flexor reflex was recorded at the intervals indicated and the d crease of the flexor reflex is given as a function of stimulus strength expres ed as multiples of threshold for the HS (B) and GS (D) nerves respectively

afferents at 1 82 and at 2 times threshold caused a considerable increase in excitability (cf Eccles *et al* 1963b) It has b en regularly observed that group I afferent volleys have no action on contralateral cutaneous fibres

Fig 5 was from the same experiment as Figs 3 and 4 but for the interactions between the ankle and toe extensors PL-FDHL and the contralateral Sur nerve The results closely resemble those when conditioning of HS was employed as in Figs 3 and 4 or when conditioning by volleys in a pure flexor nerve the knee flexor PBST or ankle flexor DP was used The variation in the right hand graph (Fig 5) indicating a de crease at low threshold is within the error of measurement Thus only volleys in group

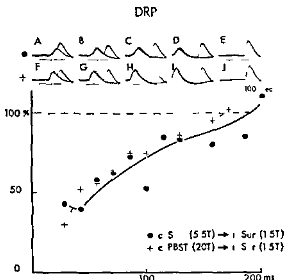


Fig 7 Interaction of dorsal root potentials recorded from the same root filament Record E shows the test DRP alone evoked by a s ngle volley in the ipsilateral Sur nerve Records A D show the DRPs evoked by the ipsilat eral Sur volleys superimposed on the DRPs evoked by the cond tioning contralateral Sur volleys The t me course for the decrease of the contralateral Sur DRPs is plotted in the graph (●) In re cords F I are s mil rly illustrated th interactions between the DRPs evoked from ipsilateral Sur and contralateral PBST nerves J being the DRP evoked by ipsilateral Sur alone The full time course is plotted (+) in the graph

II and III muscle afferents were effective in increasing the excitability of the contra lateral cutaneous afferents The increase in excitability appeared to depend only on the number of high threshold nerve fibres activated and was independent of the func tional role of the muscle whether flexor or extensor

Inhibition of transmissi n i cutaneous pathways

It has been established that the depolarization of the ipsilateral presynaptic afferent terminals is matched by a corresponding depression of reflex responses evoked by impulses in the fibres being depolarized (Eccles Kostyuk and Schmidt 1962b) On stimulation of the sur l nerve the flexor reflex discharge was recorded in the S1 ventral root The integrate i i l of this flexor reflex was measured at various intervals after conditioning volleys were applied to the contralateral HS nerve (Fig 6A) At a condi tioning stimulus strength activating group I and most of group II fibres (at 4 2 times threshold) there was an early increase in the reflex discharges matching the post synaptic excitation exerted on the motoneurones This fac litation passed into a small depression of the flexor reflex discharge of about 10 The depression increased to 25 % when the group III fibres v ere activated at 10 5 times threshold (□) and to 50 at 20 times threshold (Fig 6A B) Depending on the stimulus strength used the optimal effect appeared at 35—70 msec there being a progressive delay of the maximum as the stimulus was strengthened Therefore for examining the depression produced by a gradually increasing conditioning stimulus strength two fixed intervals were chosen one at 39 msec for disclosing threshold action and one at 58 msec for better comparison of th action added by activity in higher threshold fibres as illustrated in Fig 6B Volleys in contralateral group I muscle afferents did not e citable act on Group II and III volleys have been found to evoke IPSPs in flexor motoneurones but the duration of these postsy never found to exceed 50 msec (Holmqvist and Lundberg

It is not known how much of this depression illustrated in Fig 6 is exerted on the interneurones of the test reflex pathway

Similarly Fig 6C D illustrates the action of volleys in the ankle extensor CS onto the contralateral flexor reflex evoked by stimulation of the Sur nerve In general good agreement was found between the depression of the flexor discharge whether produced by conditioning volleys in contralateral muscle nerves or in skin nerves The latter tended to be slightly more effective but evoked much less depression than volleys in ipsilateral skin or muscle nerves (Eccles Kostyuk and Schmidt 1962b) These quantitative differences in the flexor reflex depression produced by ipsilateral and contralateral nerve volleys are *greater than the discrepancies between* corresponding ipsi and contralateral I ADs However in this connection it should be remembered that ipsilateral conditioning volleys themselves evoke marked flexor reflex discharge and consequently cause refractoriness of neurones involved in the testing pathway On the other hand contralateral volleys evoke only very small ventral root discharges

The DRP evoked by a cutaneous volley (ipsilateral Sur at 1 5T records F J Fig 7) was used to test the depression evoked by one volley in the contralateral PBST nerve and is plotted as crosses in Fig 7 The depression lasts for about 200 msec This depression to 50 % of the control is of the same order of magnitude as that evoked by a stimulus (5 5 times threshold) applied to the contralateral Sur nerve (Fig 7A D) The maximal depression was probably due to two factors interneuronal depression as well as presynaptic inhibition i e diminished impulse *transmission in the cutaneous* nerve terminals due to depolarization

Discussion

The results reported here are a link in a series of previous papers on the pattern of organization of presynaptic inhibition (Eccles Eccles and Magni 1961 Eccles Kostyuk and Schmidt 1962a Eccles Magni and Willis 1962 Eccles *et al* 1963a b)

The DRPs evoked by volleys in the contralateral muscle nerves reflect the combined action onto all filaments in the rootlet recorded from Consequently a demonstration of crossed DRPs only supports the results obtained with other methods where the depolarization onto cutaneous fibres could be specifically tested However the DRPs matched the intracellularly recorded PADs and the increased intraspinal excitability of the cutaneous fibres Therefore it is permissible to conclude that the DRPs evoked by contralateral muscle volleys at least partly consisted of depolarization of the *central terminals of cutaneous fibres (cf* Eccles Magni and Willis 1962)

As the depolarization of nerve terminals has been regarded as the primary cause for presynaptic inhibition it would be expected that th observed depolarization of the cutaneous fibres in a similar way depresses cutaneous reflexes This has been tested on three cutaneous pathways 1) *the flexor reflex discharge recorded in the ventral root* 2) the DRP and 3) ascending spinal pathways Contralateral muscle volleys depressed the flexor reflex discharge and the DRP with a time course corresponding to the observed depolarization of the primary skin afferents There are also other possibilities in which the depression could be exerted Volleys in contralateral muscle nerves have evoked *crossed* inhibitory as well as excitatory postsynaptic potentials *in flexor* motoneurones but these postsynaptic potentials were small and were never *longer* than 50 msec The optimal postsynaptic action occurred at about *20 msec* whereas the presynaptic inhibitory action had a maximum at about 50—80 msec and

a duration of 150 msec Finally the observed depression of the two pathways could also have been caused earlier in the interneuronal chain In order to overcome this complicating factor the effect was tested on the monosynaptic transmission from cutaneous afferents to ipsilateral tract fibres located in the dorsolateral funiculus (Lundberg and Oscarsson 1960 1961) Stimulation of contralateral high threshold muscle afferents depressed slightly (only 5 %) the ascending monosynaptic discharge though the depression had a long duration of about 100 msec This value seems very low but the effect has been tested on a pathway with a powerful synaptic linkage and a high safety factor for transmission In view of the generally weak contralateral presynaptic actions the observed small depression does not seem out of proportion with the 25—30 % depression found when 4 ipsilateral muscle volleys were used for conditioning (Eccles Kostyuk and Schmidt 1962b)

In general the features displayed by volleys in contralateral muscle nerves onto cutaneous afferents resembled those evoked from the ipsilateral side (Eccles *et al* 1963b cf also Barron and Matthews 1935 1938 Lloyd and McIntyre 1949) However there are both quantitative and qualitative dissimilarities The contralateral effects are always much weaker and their latency is longer This discrepancy can be explained either by transmission over longer interneuronal chains or by weaker synaptic linkage hence more extensive temporal and spatial summation is required for the crossed presynaptic actions

The other important discrepancy between the actions from the two sides concerns the pattern of organization Cutaneous fibres are depolarized by volleys in ipsilateral group Ib II and III afferents from muscles (Eccles *et al* 1963b) while only group II and III volleys from contralateral muscles evoked any presynaptic depolarization

In the spinal animal the crossed presynaptic effects from muscle nerves onto cutaneous pathways are weak and their physiological significance is difficult to estimate However not less than three supraspinal centres are supposed to influence the PADs in groups Ib II and III muscle afferents and cutaneous afferents namely (1) dorsal midline structures in the brain stem (Carpenter Engberg and Lundberg 1962) (2) ventral more widespread regions of the brain stem (Carpenter *et al* 1962) (3) and finally from sensorimotor cortex (Andersen Eccles and Sears 1964 Carpenter Lundberg and Norrsell 1963) It is of special interest that the latter pathway evoked bilateral actions also after transection of the corpus callosum (Andersen *et al* 1964) or after hemisection of the spinal cord (Carpenter *et al* 1963) thus indicating crossed connections at the spinal level There is evidence that the supraspinal influences are exerted onto interneurones mediating local spinal presynaptic reflex actions Therefore it is possible that crossed presynaptic actions could be enhanced by supraspinal systems

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Ascending Systems of Catecholamine Neurons from the Lower Brain Stem

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With the help of the histochemical fluorescence method developed by Falck and Hillarp it has been possible to demonstrate and map out 3 systems of specific neurons which form and store dopamine (DA) noradrenaline (NA) and 5-hydroxytryptamine (5 HT) respectively one nigro neostriatal DA system (Andén *et al* 1964 a and b) and two bulbo spinal NA and 5 HT systems (Carlsson *et al* 1964 Dahlström and Fuxe 1964) These neurons have low concentrations of the amines in their cell bodies and extremely high concentrations in their synaptic terminals There is good reason to believe that the specific monoamine neurons in the brain act by releasing the amines from their terminals (Andén *et al* 1964 c Dahlström and Fuxe 1964) Abundant catecholamine (CA) terminals however exist in many regions of the brain especially those belonging to the limbic system (Fuxe 1964 a) and the hypothalamus (Carlsson *et al* 1962) Since practically all the cell bodies of the CA neurons seem to be localized to the lower brain stem it is probable that the terminals in the forebrain arise from fibre systems ascending from these cell groups Such systems have in fact been found e g in the medial forebrain bundle (Dahlström and Fuxe 1964) In the present paper direct experimental evidence is given for this view

In about 30 male albino rats (body wt 150 to 200 g) large electolytic lesions (see Hillarp 1947) were made unilaterally in the ventral half of the cranial part of the mesencephalon in order to destroy all the CA cells in this region The animals were killed 8 to 12 days after operation together with 10 normal animals which served as controls The medulla oblongata pons-mesencephalon diencephalon and the left and right half of the telencephalon up to a transverse plane passing through the most anterior portion of the neostriatum were dissected out, freeze dried treated for demonstration of primary CA and embedded in paraffin as described in detail previously (Dahlström and Fuxe 1964) Transverse serial sections (8 to 10 μ thick) were prepared for fluorescence microscopy

Most of the CA nerve cells in the lower brain stem are localized to 3 large groups A8 to A10 in the mesencephalon Dahlström and Fuxe (1964) In 10 of the operated animals these groups were practically completley destroyed unilaterally while the cells on the other side seemed to be for the most part unaffected The CA terminals within the brain rostrally — but not caudally (including the spinal cord) — to the lesion showed on the operated side a large decrease in number in many regions, especially the area preoptica the nuc dorsomedialis hypothalami the nuc paraventricularis the

amygdaloid cortex and the hippocampal formation In some areas they had practically disappeared, e g tuberculum olfactorium nuc accumbens neostriatum Some areas however showed less reduction (nuc *supraopticus nuc interstitialis striae terminalis*) or no obvious decrease (the external layer of the median eminence *nuc periventricularis* rotundocellularis) In the animals with less complete destruction of the cell groups a much less marked decrease was observed in the number of CA terminals The terminals on the contralateral side may have been reduced to some extent but no marked decrease was seen

Of the 3 mesencephalic groups the A9 cells present in the substantia nigra belong to the nigro-neostriatal DA system It therefore seems that the CA cells within the reticular formation of the mesencephalon and in the basal medial part surrounding the nuc interpeduncularis (A8 and A10 respectively) give rise to most of the ascending axons from which the CA terminals in the forebrain are derived These ascending fibre systems also seem to a large extent to be uncrossed It is possible however that some of the fibres cross over to the other side at the site of the lesion — The fact that the very abundant CA terminals around the primary plexus of the hypophyseal portal system in the median eminence appeared unaffected supports the view that they belong to tuberoinfundibular CA neurons (Fuxe 1964 b)

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Some Morphological Features of Catecholamine Storing Nerve Vesicles

By

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Subcellular particles rich in noradrenaline have been obtained from homogenized bovine splenic nerves (Euler and Hillarp 1956 Schumann 1958) Preliminary electron microscopic studies (Bahr and Euler 1957 Bloom and Euler 1957 unpublished observations) indicated that the particles were stainable with osmium and had a diameter ranging from 0 03 to 0 2 μ In many instances a notch on the spheric particle was noted

Using the technique of negative staining with phosphotungstic acid (final concentration 1 per cent of the sodium salt) it was possible to verify the existence of a population of granules with diameters 0 03—0 1 μ and a few occasional larger ones in the sediment from a suspension of particles from the press juice of bovine splenic nerves The particles appeared as rings (Fig 1) some of them vesicle like and filled with the contrast medium while others appeared as solid bodies A characteristic feature possibly of significance for the intraaxonal arrangement of the particles is the occurrence of the previously observed notches which with the negative staining technique were much more obvious On many occasions the notches were extended to handle shaped or tail like structures mostly extending from opposite ends of the granules Frequently the vesicles occurred in pairs and sometimes even in short chains (Fig 1 c)

The appearance of the vesicles in chain like or pearl string like structures is suggestive of a system similar to that described for the occurrence and formation of granules in the Golgi apparatus (Scharrer and Brown 1961) This might mean that the granules are arranged in a kind of network or lattice in the axon and presumably in the terminals The adrenergic nerve endings shown in Fig 10 and 13 in Richardson s paper (1962) show a somewhat similar arrangement of the vesicles in sequences covered by a kind of tubular membrane

A full account of the results will appear elsewhere

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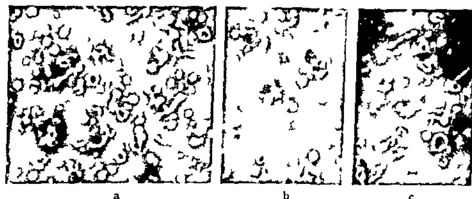


Fig 1 Isolated transmitter vesicles from press juice of bovine splenic nerves in phosphate buffer pH 7 5 *sedimented at 50 000 × g and resuspended in 0 07 M phosphate buffer and* 1 per cent sodium phosphotungstate pH 7 5 Inserted mark = 0 1 μ

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The Effect of Asphyxia on Induced Cortical Activity in Fetal Sheep

By

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In earlier studies on the development of the electrocorticogram (ECG) in fetal sheep it was found that the spontaneous cortical activity appears at a gestational age of about 65 days (full term about 150 days) and consists of trains of spindle shaped waves of a frequency of 8—12 c/sec (Bernhard *et al* 1959) During *asphyxia* induced by clamping the umbilical cord the fetal ECG is blocked after about 2 min and in this respect the fetus does not differ from the adult animal (Bernhard and Kolmodin 1962) In contrast the cortical positive steady potential (SP recorded between the intact cortical surface and inactive tissue) is remarkably resistant to asphyxia in the immature brain whereas in adult animals under the influence of asphyxia the SP decreases and turns negative within a few minutes The ratio between the fetal SP-change caused by asphyxia and that of the adult animal is about 1 5 (Eidelberg *et al* 1964 a) The fact that the SP has been observed as early as at a gestation age of 30 days (weight 1—2 g) indicates that it represents a relatively primitive function The above observation led to the conclusion that at least during asphyxia this primitive function can be maintained by anaerobic energy sources in contrast to functions represented by the ECG which also in fetuses seem to depend mainly on the supply of oxygen.

In the present investigation the study of the effect of asphyxia on the fetal brain was extended to electrical signs of activity which like the ECG reflect the neuronal activity more directly than the SP Two such cortical functions have been chosen namely the *direct cortical response* (DCR) and the *transcallosal response* (TCR) which are considered to be generated by partly similar cortical structures and which both can be recorded at the same stage of fetal maturation i e at a fetal age of about 65 days corresponding to a weight of 100—150 g (Eidelberg *et al* 1964 b Meyerson 1964) The experimental series comprises 6 fetal sheep of ages from 95 to 150 days (weight 600—2 000 g) Pregnant ewes were chosen at certain stages of the gestation period and their fetuses were delivered by cesarean section In the unanesthetized fetuses the umbilical circulation was left intact The older fetuses were immobilized (Flaxedil) to prevent spontaneous breathing Asphyxia was induced by clamping the umbilical cord

While the TCR in fetuses was readily affected and totally blocked within about 1—2 min the DCR was found to be far more resistent to asphyxia and failure of circulation In the youngest fetuses studied (95 days) the DCR could still be obtained

after 12—14 min of cord clamping i e at a time when the heart activity was greatly reduced In somewhat older fetuses (about 115 days) the response disappeared after 8—10 min and in those near term after about 6 min In control experiments on adult sheep the DCR disappeared even after 2—3 min of asphyxia (cf Chang 1951) It should be mentioned that the configuration of the DCR is the same in the fetus as in the adult sheep These preliminary experiments indicate that the resistance to asphyxia decreases with increasing fetal age No constant changes of the response were observed but towards the end of the period of asphyxia the stimulus threshold tended to increase

The resistance of the immature sheep brain to asphyxia as expressed by the behaviour of the DCR may be a characteristic which to a certain extent is bound to intra uterine life and not only to the stage of functional and morphological maturation This view may be favoured by the fact that also at the end of the gestation period the fetal DCR is significantly more resistant to asphyxia than is this response in the adult animal although the brain of the full term sheep is relatively mature both anatomically and functionally

The results of the present investigation indicate that the fetal direct cortical response — which can be regarded as a more direct manifestation of the cortical excitability than the steady potential — can be maintained also by energy generated by anaerobic metabolism The fact finally that in the immature brain the direct cortical response is far more resistant to asphyxia than the transcallosal response — which in all probability is of postsynaptic origin (see e g Grafstein 1959) — should be taken into account in the discussion concerning the functional backgrounds of these responses

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On the Presence of a Second Specific Cell System in Mammalian Thyroid Gland

By

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It has recently been found that monoaminergic mechanisms play a role in the function of the endocrine cells in the pancreas (Falck and Hellman 1963 1964 Cegrell *et al* 1964) This prompted an investigation on other endocrine organs and in this paper some pre liminary results from studies on the thyroid gland are reported

Sheep cat rabbit guinea pig rat and mouse were used in these experiments The direct cellular localization of the monoamines was performed by exposing freeze-dried tissue to formaldehyde gas according to Falck (1962) The content of monoamines in the thyroid gland was determined spectrophotofluorimetrically according to Bertler *et al* (1958) and Bertler and Rosengren (1959)

In the *sheep* thyroid the formaldehyde treatment developed the intense green fluorescence characteristic for primary catecholamines in some nerve fibers and in a relatively small number of granular cells Using the Astra blue staining (Bloom and Kelly 1960) the latter were identified as mast cells which in ruminants are known to carry dopamine (Falck *et al* 1964) Most of the nerve fibers had the typical appearance of terminal sympathetic fibers (*cf* Falck 1962) and seemed to be related to the vessels A yellow fluorescence characteristic of certain tryptamine derivatives developed in a large number of cells which either appeared in clusters between the follicles or were located in the follicular epithelium The latter showed no fluorescence except for a weak orange autofluorescence in a granular material posited in the apical part of the cells as reported previously (Sjostrand 1944) In agreement with the fluorescence microscope findings the fluorimetric determinations demonstrated the presence of small amounts of dopamine (0 13 μg/g) and a high content of 5 HT (1 7 μg/g) whereas neither noradrenaline nor adrenaline was detected The yellow fluorescent cells had a markedly uneven distribution in the gland being very sparse or almost absent in some areas especially in those containing the largest follicles The fluorescent material was found only in the cytoplasm and seemed to be confined to very fine granules which were smaller than the green fluorescent granules in the mast cells Many of the cells were provided with processes which in most cases seemed to run contiguous to the base of the follicular epithelium

No fluorescent cells except the 5-HT containing mast cells in rat and mouse were detected in the thyroid gland of the other species. However, experiments in progress have disclosed a cell system in *rat* and *mouse* similar to that in the sheep thyroid. This cell system, in contrast to the follicular epithelium proper, is able to take up and for a limited time store injected catecholamines and 5-HT; further it may contain both monoamine oxidase and the decarboxylating enzyme for DOPA and 5-hydroxytryptophan. These cells have mostly a parafollicular localization but often lie in the epithelial lining of the follicles. In rat they were found unevenly distributed over the gland, but in the *mouse they have a peculiar localization being accumulated in the dorso-medial part* from approximately the level of the first tracheal ring up to the lower border of the cricoid cartilage.

The results thus demonstrate that the so-called parafollicular (interfollicular, interstitial) cells, or at least their main part, differ in major respects from the follicular cells and thus might constitute a second specific cell system in the thyroid gland. The work of Wissig (1962) supports strongly this assumption. He found electron microscopically a fine structure of parafollicular cells that differ considerably from the follicular cells, chiefly because the parafollicular cells have a rich content of small spherical vesicles. He concluded that these cells *are not related to follicular cells but are probably a second independent class of endocrine epithelium.*

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Depletion of the Amine Stores in Brain Catecholamine Terminals on Amygdaloid Stimulation

By

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Intermittent stimulation for 3 hrs within the amygdaloid nuclei in unrestrained cats which produces repeated outbursts of sham rage (*cf* Hilton and Zbrozyna 1963) has been found to result in lowered brain levels of noradrenaline (NA) but not of dopamine (DA) (Gunne and Reis 1963) There is little doubt that these amines are present within specific DA and NA neurons (*cf* Dahlstrom and Fuxe 1964) The fine synaptic terminals of these neurons show abundant small enlargements varicosities which store DA and NA in very high concentrations (Carlsson Falck and Hillarp 1962 Fuxe 1964)

In the present paper it is shown by the histochemical fluorescence method of Falck and Hillarp that the amine stores in many systems of catecholamine (CA) terminals are depleted on amygdaloid stimulation especially if resynthesis of the amines is inhibited The inhibition was produced by administration of H 22/54 (*a* propyl 3 4 dihydroxyphenylacetamid) a potent inhibitor of the first step in the CA synthesis (Carlsson Corrodi and Waldeck 1963)

Twelve adult cats (b wt 2 0—4 0 kg) were used Stainless steel needle electrodes were implanted into the region of the basomedial amygdaloid nucleus Intermittent stimulation was performed as described by Gunne and Reis (1963) In 4 cats CA synthesis inhib tor H 22/54 100 to 200 mg/kg was administered i p 15 min before the start of stimulation In 3 cats administration of this drug (200 mg/kg) was made without subsequent stimulation and in 2 cats amygdaloid stimulation was performed without inhibition of the CA synthesis The rest of the cats were used as controls

The hypothalamus the preoptic area the septal area the amygdaloid cortex the nuc caudatus putamen the ventral part of the mesencephalon and the ventricular part of the medulla oblongata were dissected out freeze-dried treated with formaldehyde gas for 1 hr embedded sectioned and mounted as described previously (Dahlstrom and Fuxe 1964)

The CA terminals in practically all regions of the forebrain (for instance in the nuc *dorsomedialis* hypothalami the nuc paraventricularis the median eminence the retrochiasmatic area nuc arcuatus nuc preopticus suprachiasmaticus nuc pre

opticus medialis and the ventral part of the nuc interstitialis striae terminalis) showed *a marked to very marked CA depletion if amygdaloid stimulation* was combined with synthesis inhibition especially with the higher dose The cell bodies of the CA neurons in the mesencephalon however showed no obvious decrease in their amine contents and the CA terminals in the lower brain stem e g in the dorsal motor nuc of the vagus showed much less marked depletion The depletion was only observed in the varicosities which often became very weakly fluorescent with intensity levels approaching those of the thin fibre segments betwen them The varicosities belonging to any given terminal seemed to react uniformly Amygdaloid stimulation in cats which were not pretreated with H22/54 resulted in much less marked changes When H22/54 was administered without concomitant stimulation there were no changes in the regions investigated except for a fairly marked reduction of the fluorescence intensity within the nuc caudatus putamen Such a reduction within the DA terminals of this nucleus following synthesis inhibition alone probably indicates a rapid turn over of DA in this region

The present report is the first direct demonstration of adrenergic activity at synaptic levels within the central nervous system The depletion of the CA terminals usually became evident by histochemical fluorescence technique only when the CA synthesis had been inhibited This indicates that resynthesis is of high importance in the maintenance of the catecholamine levels at synaptic sites

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